TAXONOMY AND PHYLOGENY OF CRUSTOSE BROWN ALGAE (PHAEOPHYCEAE) FROM MALAYSIA AND LOMBOK ISLAND, INDONESIA

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ABSTRACT

Studies on crustose brown algae are relatively few despite a long history of studies conducted since the 1800s, with temperate species forming the bulk of these studies. There is a need for more focus on crustose brown algae particularly in the tropics as they are generally different from those in the temperate regions. These algae received little attention due to their simple appearance (brownish-black spots on rocks) and lack of apparent economic value despite some being the dominant flora of certain habitats. Taxonomic confusion arising from morphological simplicity largely dependent on the reproductive structures and overlap in morpho-anatomical features among species necessitates the use of molecular approach. This study aims to enhance our understanding of the taxonomy and phylogeny of these understudied algae in Malaysia and Lombok Island by combining morpho-anatomical observations and molecular sequence data [plastid rubisco large subunit (*rbc*L) and partial mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1-5') gene sequences]. Key diagnostic morphological characters for identification include relative thickness of the crust, assembly of erect filaments, number of chloroplast(s) and most importantly the organisation of reproductive structures. Results indicated that the common crustose brown algae in Malaysia and Lombok Island are species of *Mesospora*, *Diplura* and *Neoralfsia expansa*. Seven species of *Mesospora* were identified in the present study of which three were formally described (*M. schmidtii*, *M. negrosensis* and *M. elongata*) and four are putative new species to be described (*Mesospora* sp. C, *Mesospora* sp. D, *Mesospora* sp. 1 and *Mesospora* sp. 2). Partial output from this study leads to the recent description of *Mesospora elongata* Poong, Lim & Phang from Japan and Lombok Island and a new record of *M. negrosensis* for Malaysia. The presence of *M. elongata* in Malaysia is reported here for the first time since the initial publication of this taxon.

Mesospora sp. C and *M. schmidtii* were reported earlier in Malaysia. This study also marks the first report of *Mesospora* sp. D (previously reported in Japan) and four putative species of *Diplura* for Malaysia and Indonesia. The taxon provisionally named "*Mesospora*" sp. 3 and the closely related *Mesospora* sp. G are likely members of a new genus. Other crustose brown algae examined in this study include a *Ralfsia*-like species and two yet-to-be identified taxa. The crustose brown algae are polyphyletic and occur in widely divergent clades among the brown algae with the majority placed in Ralfsiales while the rest are closely associated with other phaeophyceaen orders such as Sphacelariales and Ishigeales. Circumscription of Ralfsiales comprises the Ralfsiaceae, Neoralfsiaceae, Mesosporaceae and a possible new family to accommodate "*Mesospora*" sp. 3. Establishment of a new family for members of the genus *Diplura* is underway pending the collection of the generitype, *Diplura simulans*. Molecular analyses incorporating *Hapalospongidion saxigenum* indicate that *Mesospora*, currently synonymised with *Hapalospongidion* should be retained as a separate genus. The *rbc*L marker performed better in the elucidation of phylogeny at higher classification levels, whereas *cox*1-5' is more suited as a barcoding marker for species level identification of the crustose brown algae.

ABSTRAK

Kajian mengenai alga perang berkerak agak terhad walaupun kajian tersebut telah bermula sejak abad ke-19 dan kebanyakannya melibatkan spesies dari kawasan beriklim sederhana. Tumpuan yang lebih berat perlu diberikan terhadap alga jenis tersebut, khasnya di kawasan tropika kerana ianya adalah berbeza daripada mereka di kawasan beriklim sederhana. Alga-alga ini kurang menerima perhatian disebabkan rupanya yang biasa (tompok perang-kehitaman di atas batuan) dan tiadanya kepentingan ekonomi yang ketara, meskipun sesetengahnya merupakan flora yang dominan di beberapa habitat. Kekeliruan taksonomi berikutan morfologi yang biasa dan bergantung berat terhadap struktur reproduktif serta pertindihan dalam ciri-ciri morfologi dan anatomi di antara spesies memerlukan penggunaan pendekatan molekular. Kajian ini bertujuan meningkatkan pemahaman terhadap taksonomi, filogeni dan floristik bagi alga perang berkerak di Malaysia melalui penggabungan data molekular [jujukan gen *plastid rubisco large subunit* (*rbc*L) dan *mitochondrial cytochrome c oxidase subunit 1* (*cox*1) separa] dan pemerhatian morfo-anatomi. Karakter morfologi diagnostik yang utama untuk pengenalpastian merangkumi ketebalan kerak secara relatif, struktur bina filamen terdiri, nombor kloroplas dan yang paling mustahak organisasi struktur reproduktif. Keputusan menunjukkan bahawa alga perang berkerak yang biasa dijumpai di Malaysia dan Lombok terdiri dari spesies *Mesospora, Diplura* dan *Neoralfsia expansa.* Tujuh spesies *Mesospora* dikenalpasti dalam kajian ini yang mana tiga telah dihuraikan secara formal (*M. schmidtii, M. negrosensis* dan *M. elongata*) dan empat berkemungkinan merupakan spesies baru yang perlu dihuraikan (*Mesospora* sp. C, *Mesospora* sp. D, *Mesospora* sp. 1 dan *Mesospora* sp. 2). Sebahagian hasil kajian ini menjurus kepada penghuraian *Mesospora elongata* Poong, Lim & Phang dari Jepun dan Lombok dan rekod baru *M. negrosensis* bagi Malaysia. Kehadiran *M. elongata* di Malaysia

dilaporkan di sini buat julung kali sejak penerbitan pertama spesies ini. *Mesospora* sp. C dan *M. schmidtii* telah dilaporkan di Malaysia sebelum ini. Kajian ini juga merupakan laporan pertama *Mesospora* sp. D (sebelum ini dilaporkan di Jepun) dan empat spesies *Diplura* bagi Malaysia dan Indonesia. Spesies yang buat sementara dinamai "*Mesospora*" sp. 3 dan *Mesospora* sp. G yang berkait rapat dengannya berkemungkinan besar merupakan ahli genus yang baru. Alga perang berkerak lain yang diselidik dalam kajian ini merangkumi satu spesies seiras *Ralfsia* dan dua taksa yang belum dikenalpasti. Alga perang berkerak adalah *polyphyletic* dan hadir dalam *clades* yang amat berjauhan antara alga perang, yang mana kebanyakkannya ditempatkan dalam Ralfsiales manakala lebihannya berkait rapat dengan order alga perang seperti Sphacelariales dan Ishigeales. Ralfsiales kini merangkumi family-famili Ralfsiaceae, Neoralfsiaceae, Mesosporaceae dan sebuah famili baru bagi "*Mesospora*" sp. 3. Penubuhan famili baru untuk ahli-ahli genus *Diplura* perlu ditangguh sehingga spesimen *generitype* iaitu *Diplura simulans* diperoleh. Analisa molekular yang merangkumi *Hapalospongidion saxigenum* menunjukkan bahawa *Mesospora* yang kini dianggap sebagai sinonim *Hapalospongidion* patut diasingkan sebagai genus berlainan. Penanda *rbc*L adalah lebih sesuai bagi penghuraian filogeni pada peringkat klasifikasi yang lebih tinggi manakala *cox*1-5' lebih sesuai sebagai penanda barkod bagi pengenalpastian alga perang berkerak di peringkat spesies.

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LIST OF SYMBOLS AND ABBREVIATIONS

A	Adenine
AICc	corrected Akaike Information Criterion
BI	Bayesian Inference
BICc	corrected Bayesian Information Criterion
BP	Bootstrap Percentage
C	Cytosine
CI	Consistency Index
*cox*1-5’	5’ end of the cytochrome *c* oxidase subunit 1 gene
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
G	Guanine
ITS	Internal transcribed spacer
IUPAC	International Union of Pure and Applied Chemistry
LSU	Large subunit of ribosomal DNA
ML	Maximum likelihood
MP	Maximum parsimony
NJ	Neighbour joining
OD	Optical density
PAUP	Phylogenetic analysis using parsimony
PCR	Polymerase chain reaction
PP	Posterior probabilities
*psa*A	Photosystem I P700 chlorophyll a apoprotein A1 gene
*psb*A	Photosystem II thylakoid protein D1 gene
*psb*C	Photosystem II CP43 chlorophyll apoprotein gene
*rbc*L	*Rubisco* large subunit
rDNA	Ribosomal deoxyribonucleic acid
RI	Retention index
rubisco	Ribulose 1,5-biphosphate carboxylase/oxygenase
SEM	Scanning electron microscopy
SSU	Small subunit of ribosomal DNA
T	Thymine
TEM	Transmission electron microscopy
TBR	Tree bisection reconnection
UV	Ultraviolet
°C	degree Celcius
µg	microgram
µl	microlitre
µm	micrometre
cm	centimetre
mg	milligram
mins	minutes
ml	mililitre
mM	milimolar
ng	nanogram
nm	nanometre
pmol	picomole
secs	seconds

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CHAPTER 1: INTRODUCTION

1.1 Current scenario of taxonomy studies with emphasis on algal taxonomic effort

Taxonomy, the branch of science dealing with identification, description, naming and classification of living organisms dates back to over 250 years when Linnaeus introduced the binomial classification still used today (Linnaeus, 1758). The importance of this discipline which delivers basic and indispensable knowledge to numerous fields of human interest and underpinning a host of scientific research, include cataloguing of diversity, biodiversity conservation, understanding of ecosystem services, documenting climate change and ecological modelling (De Clerck et al., 2013). In the context of algal taxonomy studies, its importance lies mainly in correct identification for cultivation, exploitation and conservation purposes. As the anthropogenic impacts on the biota of the Earth become more apparent, with current extinction rates hitting as much as 27,000 of the known species per annum (Maddison et al., 2012), the exercise of discovering and documenting biodiversity has been given an increased sense of urgency (Cardinale et al., 2012). Joppa et al. (2011) reported that both the rates of species description and the number of taxonomists have increased exponentially since the 1950s. They also noted a marked decline in the number of species described per taxonomist, attributing this to the increasing difficulty of finding new species in an ever-declining "missing-species pool". The choice of test cases (e.g., higher plants, birds, mammals) representing well-studied and taxonomist-rich groups inadvertently lead to the decline (Bacher, 2012; Samyn and De Clerck, 2012).

On the other hand, studies (e.g., De Clerck et al., 2013) have shown that there is no evidence for a decrease in the description rates of algal species unlike those well-known groups such as the birds, mammals and higher plants in which rates decrease as

fewer species remained to be described (Joppa et al., 2011). In fact, De Clerck et al. (2013) reported a gradual overall increase of the description rate over time. However, algal taxonomists face a conundrum in the remarkable uncertainty of the taxonomic status of an immense number of algal species name, many which are described poorly in isolated publications, with no attempt to relate a new taxon to existing species and classifications. This can be seen in the huge number of synonyms and species names that are flagged as uncertain in AlgaeBase (Guiry and Guiry, 2013) which databases taxonomic, nomenclatural, bibliographic and biogeographical information on micro- and macroalgae on a worldwide basis. A major challenge in algal taxonomy is the relatively few diagnostic morphological characters combined with high levels of convergence (several cryptic species) or divergence (a single species) which may have resulted from various environmental factors. Apart from difficulty in identification, this has inadvertently led to various circumscriptions based on subjective interpretation rather than formal analyses (Draisma, 2002).

Advances in culture techniques, analytical biochemistry (e.g., pigment composition), microscopy (TEM and SEM to visualise cellular ultrastructure details) and essentially gene sequencing technology (molecular phylogenetics, molecular taxonomy) which enabled a sudden rise in the number of algal classes from 1990 onward, has provided us with an improved understanding of the deep divergences separating many algal groups (De Clerck et al., 2013). Algal systematics has been revolutionised following the sequencing of target genes in individual organisms or the more recent environmental sequencing (e.g., Rusch et al., 2007).

As molecular data become more and more accessible, gene sequences have been employed in systematic studies to complement the use of morphological characters which has its own limitations. Two approaches, i.e. DNA taxonomy in which species are delineated based on sequence data using evolutionary species concepts (Vogler and

Monaghan, 2007) and DNA barcoding which identifies specimens based on sequence similarity against a database of a priori defined species (Hebert et al., 2003) are now widely used by phycologists to discover new species and assess species-level diversity (e.g., Saunders, 2005; Cianciola et al., 2010). However, a number of these sequences are not linked to a proper Latin binomial (consisting of a genus name and specific epithet) and are believed to be a result of two interlinked factors. First, describing species is a tedious and time-consuming process as opposed to the significantly faster speed of generating sequences and hence the discovery of diversity. The second factor concerns the mismatch between genetic diversity and morphology and the fact that morphology does not necessarily reflect species boundaries. This becomes more of an issue when dealing with organisms of diminishing morphological complexity and size (e.g., Kain et al., 2010). Furthermore, in cases where cryptic diversity is rampant and morphology is therefore inadequate to properly identify specimens, it is virtually impossible to link specimens to existing names. In other words, taxonomists are held back from associating gene sequences to published names due to this uncertainty. This may in part, lead to a tendency, in phycology, to gradually shift from species-base identifiers to a more informal system of using clade-, specimen- or strain-based identifiers to communicate biological information (e.g., Zuccarello and West, 2003).

Godfray (2002) aptly described a major stumbling block in taxonomic effort when he wrote "In some taxonomic groups, the past acts as a dead weight on the subject, the complex synonymy and scattered type material deterring anyone from attempting a modern revision". Although it would be ideal for the type of every single species, currently accepted species as well as the synonyms and species of uncertain status to be sequenced; this may not be possible for a few reasons. Types may be inadequate or unavailable, or their condition does not permit extraction of DNA (e.g., formalin preserved specimens, microscope slides, drawings, fumigated herbaria;

Saunders and McDevit, 2012). Tautz et al. (2003) suggested for experienced taxonomists to identify newly collected specimens that could be used for DNA extraction and subsequently designated as a neotype to overcome the aforementioned problem. In phycology, the magnitude of the problem is further heightened by the fact that many algal types have been destroyed, or have not been located or even designated (Silva, 2008).

Various imperative measures have to be taken by all parties involved to ensure a coherent and stable taxonomic framework and thus improve the efficiency of taxonomy. Collections and herbaria can play a role by locating type material and making it available online and for sequencing (De Clerck et al., 2013). Additionally, advances in technology in terms of speed and success rate of generating representative DNA sequences from type specimens is much needed (Särkinen et al., 2012; Saunders and McDevit, 2012). De Clerck et al. (2013) mooted the idea of compulsory registration of names of new taxa which will provide a mechanism that would help solve problems in determining which names are effectively published, reducing the publication of invalid taxa and assuring that data are available online to the entire scientific community immediately on publication. They added that registration should also be extended to the large number of previously published names with uncertain status. A continued need for carefully curated DNA databases from specimens correctly identified by specialist taxonomists, and a concerted effort from taxonomists, bioinformaticians and molecular systematists is essential in DNA taxonomy (Vogler and Monaghan, 2007).

Nonetheless, DNA-based taxonomy is not without its limitations. The power of DNA sequences for identifying species is limited when species pairs have very recent origins. For some time after the initial split, new sister species will share alleles, either due to ongoing gene flow, or because of recent ancestry. In such cases, sequences from one or few individuals will not be sufficient for an unequivocal assignment to a

particular group. Insufficient taxonomical and geographical sampling may cause artificial cluster formation; clusters may collapse once closely related species are included and geographic variation is fully quantified (Moritz and Cicero, 2004; Vogler and Monaghan, 2007). There is also a complication when organelle-derived gene sequences are used as the organelles can occasionally be transferred, at least between closely related species and female-limited mode of inheritance (in the case of mitochondria). This could result in different diagnoses, depending on whether a sequence from the nuclear genome or organelle genome is used. In any case, it seems advisable to use more than one sequence region (which can also increase confidence levels in the nodes of phylogenetic trees) for assigning taxonomic status. Nevertheless, a DNA-based taxonomy system carried out in a way that integrates the strengths of the traditional morphology-based system will allow us to make full use of, and indeed requires the invaluable knowledge that has been accumulated over the centuries. This system coupled with phylogenetic information is expected to help us out of the current taxonomy crisis and would give a new impetus to biodiversity research, complementing many other ongoing efforts (Tautz et al., 2003). Furthermore, the independence of sequence data from morphology also provides a basis from which to evaluate both the diagnostic and phylogenetic value of particular morphological characters (Draisma, 2002).

1.2 Importance of phylogenetic studies

The fundamental importance of phylogenetic studies is to provide insights into organismal relationships and evolution. For the brown algae, molecular phylogenies have revealed the Schizocladiophyceae, Phaeothamniophyceae and Xanthophyceae to be their closest relatives (Potter et al., 1997; Bailey et al., 1998). At a lower taxonomic hierarchy, the phaeophycean order Ectocarpales otherwise known as the “simple brown

algae" was long thought to be the most primitive brown algae due to their simple filamentous construction (De Reviers and Rousseau, 1999) but phylogenetic studies have since shown that the Ectocarpales is not an early diverging clade and was actually derived (Phillips et al., 2008). Improved concepts of relationship based on phylogenetic analyses are also resulting in revised classifications in many groups of algae (e.g., Lim et al., 2007; Silberfeld et al., 2011)

In addition to that, phylogeny is studied for its vast applications and its links to other disciplines. Phylogenetic trees which constitute a hypothesis of the genealogical relationships of the taxa under study are able to facilitate interpretation of the evolution of diverse characters (molecular, physiological and genetic). These include investigations of biosynthetic and developmental pathways, natural products chemistry, origins and migrations of evolutionary lineages, and conservation (Daly et al., 2001). Phylogenetic hypotheses have become the framework for the choice of 'model organisms' in genomic analyses and more and more molecular biologists are using phylogenetic trees to guide their sampling of taxa for comparative research, genetic improvement of economically important species and cross-breeding. The term "reciprocal illumination" is used to describe the process of using the phylogenetic tree to test the notion that a character reappearing independently is not really the same (i.e. evolutionary homologous) despite its apparent similarity (Daly et al., 2001). Apart from that, the application of evolutionary theory to species circumscription will, in turn, shed light on certain issues in DNA taxonomy, for instance sampling strategy and the choice of suitable gene regions (Vogler and Monaghan, 2007).

As part of conservation efforts, knowledge of phylogeny can be applied to better understand differential species' phenological response to climate change which poses one of the most significant threats to biodiversity. Species' decline may not be random or uniform, but rather biased against certain clades (i.e. phylogenetic selectivity) even

between geographically disjunct communities. This information can then be used to predict which species face a greater risk of extinction as climate change is exacerbated (Davis et al., 2010). In addition to studying the impacts of climate change on biodiversity, phylogeny is also relevant to various areas of ecology (Webb et al., 2002; Vamosi et al., 2009), for instance patterns of community assembly (Cavender-Bares et al., 2006), pathogen-host interactions (Gilbert and Webb, 2007) and ecosystem function (Cadotte et al., 2008). Phylogenetic information can also contribute to conservation in a different approach. Floristic work, built on taxonomy and phylogenetic analyses are used to identify centres of endemism and of diversity, as well as rare and/or threatened species. In other words, phylogenetic studies help us to identify and conserve areas with the highest genetic and hence taxa diversity. By incorporating molecular data sets, phylogenetic analyses can help guide the protection of evolutionary processes by illuminating historical migrations and geographic and ecological origins (Daly et al., 2001).

In the field of palaeontology, phylogenetic analysis plays a role in fossil reconstruction through the inference of unpreserved attributes of extinct fossil taxa based on the cladistics distribution of known features in both the extant sister group of the taxa and more distantly related clades (Bryant and Russell, 1992).

1.3 The crustose brown algae

The crustose brown algae are a polyphyletic group of the Phaeophyceae (Lim et al. 2007), largely unnoticed in this region due to their simple appearance (brownish-black spots on rocks) and lack of apparent economic value. Abbott and Huisman (2004) remarked that these taxa have not received much attention as a result of difficulty in accessing good material. They are mostly marine, although some are reported to be

euryhaline, for instance *Porterinema fluviatile* (Waern) Porter (McCauley and Wehr, 2007) or freshwater (e.g., *Heribaudiella fluviatilis* (Areschoug) Svedelius (Stoyneva et al., 2003). This group of algae are generally epilithic, at times epizoic, and one brown crust, *Petroderma maculiforme* (Wollny) Kuckuck was reported as a lichen, forming a symbiosis with the marine fungus *Verrucaria tavaresiae* Moe (Sanders et al., 2004).

A strict definition of crustose brown algae includes only pseudoparenchymatous species, excluding those parenchymatous brown algae with prostrate thalli resembling crusts, e.g., *Sphacelaria mirabilis* (Reinke ex Batters) Prud'homme van Reine. Two genera of the Dictyotales, *Lobophora* and *Distromium*, are also crustose at times and they differ from the "actual" crustose brown algae in their life history and thallus construction. The thallus of crustose brown algae comprises a basal disc of prostrate filaments which give rise to either loosely or tightly adherent erect filaments, forming a pseudoparenchymatous crust spreading over the substrate (Fletcher, 1978; Buchanan, 2005). The ecological function of this group of algae, despite being conspicuous components of rocky intertidal and shallow subtidal communities worldwide, is relatively understudied (e.g., Williamson and Creese, 1996; Dethier and Steneck, 2001). Crustose algae are useful subjects for biogeographic studies due to their extensive distribution, slow growth rates and persistence in the event of physical and biological disturbances (Maggs, 1990).

Identification of crustose brown algae is notoriously challenging due to the limited morpho-anatomical features available and similarities between crusts of widely different taxa (Poong et al., 2014). Like the red algae, their classification and taxonomy has largely relied on presence of reproductive structures. Most are reported or hypothesised to have an isomorphic life history, which is generally the case for brown crusts in the warm water regions. On the other hand, many of those in the temperate or cold water regions are actually the alternate ploidy phase of taxa with heteromorphic

life histories, particularly members of the Scytosiphonaceae (Kain et al., 2010). Taxonomic confusion arising from morphological simplicity largely dependent on the reproductive structures and overlap in morpho-anatomical features among species necessitates the use of molecular techniques in the identification of crustose brown algal species.

1.4 Research Question

How much diversity is there in crustose brown algal taxa in Malaysia and Lombok Island (Indonesia)?

1.5 Research Objectives

The aim is to undertake a study using combination of morphological and molecular techniques to determine species diversity in Malaysia and Lombok Island (Indonesia) and to determine relationships between these species. This will be achieved by the following objectives:

a) Firstly to collect, identify and document the diversity of crustose brown algae from various localities in Malaysia and Lombok Island based on morphological and anatomical characters,

b) Secondly to generate molecular sequences and construct phylogenetic trees of the crustose brown algal specimens using the plastid encoded rubiso large subunit (*rbc*L) and the partial sequence of the mitochondrial encoded cytochrome *c* oxidase subunit 1 (*cox*1-5') genetic markers and

c) Thirdly to elucidate the phylogenetic relationship and review the taxonomic status of the various crustose brown algal taxa at the familial and ordinal levels.

1.6 Research Hypotheses

a) H_0: All morphological characters were equally reliable as diagnostic features

H_A: Not all morphological characters were equally reliable as diagnostic features

b) H_0: identification based on molecular phylogenies were coherent with morphological characters

H_A: identification based on molecular phylogenies were not coherent with morphological characters

c) H_0: phylogenies of different molecular genetic markers were congruent and have similar levels of resolution

H_A: phylogenies of different molecular genetic markers were not congruent and do not have similar levels of resolution

A schematic flowchart of the research approach of this study is outlined in Fig. 1.1.

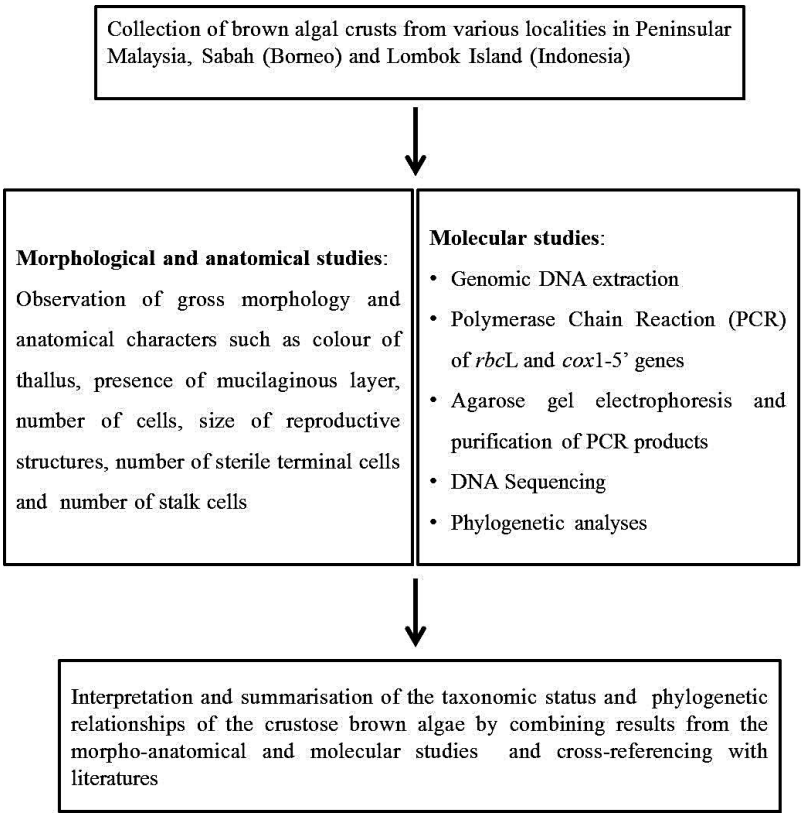

Figure 1.1 Outline of the research approach of this study.

CHAPTER 2: LITERATURE REVIEW

2.1 The Phaeophyceae

Members of the class Phaeophyceae (Kjellman, 1891[1891-1896]), numbering in approximately 285 genera (De Reviers et al., 2007) and some 1,835 species (Guiry and Guiry, 2013) are commonly known as the brown algae. They were also collectively known as the Melanophyceae (Rabenhorst, 1863) or Fucophyceae (Warming, 1884) but the name Phaeophyceae is more commonly in use at the present. The brown algae are characterised by possession of plastids derived from red algal secondary endosymbiosis and the phylum Heterokontophyta (Van den Hoek, 1978) to which they belong is subdivided in a number of classes, of which three are in close relation to the brown algae. These classes include the (predominantly) freshwater Xanthophyceae (also known as Tribophyceae) and Phaeothamniophyceae, and the marine Schizocladiophyceae with this class being treated as sister to the Phaeophyceae (Kawai et al., 2003).

The brown algae are exclusively marine in distribution with the exception of a few species from eight genera (*Heribaudiella*, *Sphacelaria*, *Bodanella*, *Pseudobodanella*, *Lithoderma*, *Porterinema*, *Ectocarpus* and *Pleurocladia*) reported to occur in freshwater with freshwater members of the last three genera being euryhaline species, inhabiting both marine and freshwater habitats (De Reviers et al., 2007). The majority of brown algae grow in the intertidal belt, extending from the upper littoral zone into the sublittoral zone, particularly of rocky shores and they attain their greatest development in terms of size, diversity and density of population in colder ocean waters in the temperate to subpolar regions of both hemispheres. Members of Fucales dominate in the intertidal belts while kelps (members of Laminariales in particular) form

spectacular sublittoral forests in these temperate areas (Papenfuss, 1951a). On the other hand, dominant brown algae found in tropical waters are mainly species of Sargassaceae and Dictyotales. Brown algae are important as primary producers in coastal marine environments, major sources of food, natural products and biomedical supplies (Andersen, 1992).

The size of the brown algae ranges from a few millimetres (e.g., crustose and filamentous forms of freshwater species such as *Pleurocladia*) to more than 60 metres in length as evidenced by *Macrocystis pyrifera* (Linnaeus) C. Agardh, the largest seaweed known. Brown algae owe their distinctive olive green to dark brown colour to the presence of carotenoid pigments, in particular fucoxanthin and violaxanthin, which masks the other pigments (chlorophylls a and c, xanthophylls, beta-carotene). Nevertheless, not all brown-coloured algae are Phaeophyceae, for the thalli of certain members of other divisions (e.g., certain species of the red algal genera *Bangia* and *Iridaea*) may appear brown and superficially resemble those of the brown algae (Papenfuss, 1951a). A number of species of the brown algae contain only one chloroplast per cell, with the majority possessing several to many chloroplasts per cell and the number of chloroplasts has been used as a taxonomic criterion (e.g., Kawai, 1991; Lim et al., 2007). The chloroplasts are usually parietal in position and they come in various shapes and forms: discoid, plate-like or ramified (Bold and Wynne, 1985). Another cytological feature with phylogenetic value is the presence or absence of pyrenoids, structures associated with the production of starch (Wynne and Loiseaux, 1976). The absence of pyrenoids are being taken as a trait of the more advanced (derived or more recently diverging) orders but the exceptions encountered in some of these advance orders seem to reduce the phyletic value of this character. The food reserve of the Phaeophyceae is known as laminarin, a soluble polysaccharide composed primarily of β,1-3 glucan (Wehr, 2002). Their cell walls contain cellulose, alginic acid

(salts of alginic acid are widely used in a variety of industries due to its emulsifying or stabilising properties) and fucoidans (Bold and Wynne, 1985).

Structure-wise, brown algal forms can be as simple as having erect, branched or unbranched filaments arising from a prostrate, filamentous basal system as exemplified by members of the order Ectocarpales and of the family Myrionemataceae in the order Chordariales (included in Ectocarpales *sensu lato*). At the other extreme, there are morphologically elaborate forms, such as members of the orders Laminariales and Fucales which have massively constructed thalli with differentiation of cells into tissues (Bold and Wynne, 1985). Three types of thallus construction are recognised which include filamentous, parenchymatous and pseudoparenchymatous. Parenchymatous tissues are also termed polystichous, pseudoparenchymatous and filamentous as haplostichous while predominantly uniseriate filaments are referred as oligostichous. Filamentous thalli consist of a single row of cells initiated from a basal, intercalary or meristematic region. On the other hand, parenchymatous thalli are derived from true tissue in which cell divisions occur in more than one plane. Pseudoparenchymatous tissues comprise closely compacted uniseriate filaments, and hence superficially resemble a true parenchyma (Draisma, 2002). Various modes of growth are displayed by the brown algae. Diffuse growth is mostly seen in the Ectocarpales *sensu lato* while the Desmarestiales, Cutleriales and some Chordariales expressed the trichothallic (localised) growth in which cell divisions are localised at the base of one or several filaments (Bold and Wynne, 1985). In the Laminariales, intercalary growth takes place via an intercalary meristem. Apical growth occurs with a single apical cell, groups of apical cells or marginal apical cells cutting off segments proximally, in the Sphacelariales, Dictyotales and Fucales (Papenfuss, 1951a; Bold and Wynne, 1985).

Generally, all orders of brown algae show an alternation of generations except for the Fucales and Tilopteridales (Papenfuss 1951a, Kawai and Sasaki, 2004). In the

Fucales, the plants are diploid and the haploid stage is represented only by male or female gametes which soon fuse to form a diploid zygote, which germinates into a diploid sporophytic plant. In other words, there is no well established gametophytic plant in Fucales, and therefore they do not show any alternation of generation (Sharma, 1986). The Tilopteridales have a virtually isomorphic or direct type of life history, which lacks sexual reproduction (Sasaki et al., 2001). In other orders of brown algae, the diploid asexual generation forms either unilocular sporangia, plurilocular sporangia or both. The unilocular sporangium is a single cell, usually spherical and enlarged, not partitioned by walls. It is the usual site of meiosis (although cases of apomeiosis have been reported), and the haploid zoospores (aplanospores in the Dictyotales) that are produced in it give rise to sexual plants. The products following meiosis can also be released as nonmotile spores or "tetraspores" (in most Dictyotales). All brown algae release motile cells at some time in their life history, either as gametes or zoospores (Bold and Wynne, 1985). The plurilocular sporangium is derived by mitotic divisions and functions as a gametangium producing haploid sexual cells when it is present on a haploid individual, or it can function as a sporangium producing diploid asexual cells when it is present on a diploid individual. The plurilocular sporangia are formed by a linear series of cells that are divided into compartments, each of which forms a single zoospore. Parthenogenetic development of unfused gametes may also take place. Depending upon the species, the gametophytes are monoecious or dioecious and they are either isogamous, anisogamous or oogamous. In isogamous and anisogamous forms, the gametangia are plurilocular sporangia. In oogamous species, a single egg is produced in each oogonium, except in certain Fucales and depending upon the species, one or many sperms in each antheridium (Papenfuss, 1951a).

2.2 Classification of the brown algae

2.2.1 Historical account on the classification of brown algae

The taxonomy and evolutionary relationships within the Phaeophyceae have always been controversial owing to the vast morphological diversity, coupled with a variety of ultrastructural and cytological characters (Draisma, 2002). Three main phases of development in the classification of brown algae may be recognised (De Reviers and Rousseau, 1999). The first phase is from the time of Areschoug (1847) to Oltmanns (1904[1904-1905]), whereby the classification is greatly influenced by the progressive discovery of the sexuality of algae, e.g., Areschoug distinguished algae with conceptacles (Cyclosporae) and without conceptacles (Episporae). From the time of Kylin (1917) to Setchell and Gardner (1925), a consistent division of the class in orders were seen. The classification was based on the type of life history (similar or dissimilar generations), the type of gamy (iso-, aniso-, oo-) and the type of spore (motile or not). The third phase can be dated from Kylin (1933) to 2001. Kylin's (1933) system of brown algae consists of the following twelve orders: Ectocarpales, Tilopteridales, Cutleriales, Sphacelariales, Dictyotales, Sporochnales, Chordariales, Desmarestiales, Laminariales, Dictyosiphonales, Punctariales and Fucales. He recognised three classes on the basis of life histories: Isogeneratae, Heterogeneratae and Cyclosporeae. The Isogeneratae possess an alternation of isomorphic generations and include five orders i.e. the Ectocarpales, Sphacelariales, Cutleriales, Tilopteridales and Dictyotales. Meanwhile, the class Heterogeneratae show an alternation of heteromorphic generations and are further divided into two subclasses Haplostichineae and Polystichineae on the basis of vegetative structure. The Haplostichineae possess thallus composed of filaments which adhere to one another, forming pseudoparenchymatous tissue and

intercalary longitudinal walls are not formed. The orders Chordariales, Sporochnales and Desmarestiales belong to this sub-class. The Polystichineae include forms in which the cells are divided by intercalary longitudinal walls resulting in true parenchymatous tissues. This subclass consists of the orders Dictyosiphonales, Punctariales and Laminariales. The class Cyclosporeae contains the single order Fucales which lacks an alternation of generations.

Fritsch (1945) and Papenfuss (1951b) rejected Kylin's classes and placed all orders in one class, the Phaeophyceae. Fritsch disagreed with Kylin's grouping because it obscures the fact that polystichous thalli also occur in the Cyclosporeae (Fucales) and the Isogeneratae (Dictyotales, Sphacelariales). Kylin's Cyclosporeae was reinstated as the subclass Cyclosporidae by Scagel (1966) followed by Wynne and Loiseaux (1976) who based their hypothesis on the different flagellation in the Fucales and Durvillaeales, all the remaining orders were placed in the subclass Phaeophycidae with the Ectocarpales being the most primitive.

Aside from establishing the order Ralfsiales, Nakamura (1972) proposed the division of brown algae into two classes, Phaeosporeae and Cyclosporeae on the basis of life history. The first class, Phaeosporeae includes brown algae with alternation of generations while the Cyclosporeae to which the Fucales belongs is without alternation of generations. In regard to the structure of the thallus, the Phaeosporeae were divided into three subclasses. The subclass Haplostichidae includes forms which possess haplostichous tissues in both sporophytic and gametophytic generations. The Polystichidae embraces forms which have polystichous tissue in both generations while the Haplopolystichidae consists forms which are haplostichous in one generation and polystichous in the other generation. Thus the subclass Haplostichidae contains the Ectocarpales, Chordariales, Ralfsiales, Sporochnales and Desmarestiales whereas the Polystichidae consist the Sphacelariales, Cutleriales, Tilopteridales and Dictyotales. The

Scytosiphonales, Dictyosiphonales and Laminariales belong to the subclass Haplopolystichidae.

The Fucales were considered an early diverging lineage that evolved independently for a long period of time, because of their peculiar life cycle. Clayton (1984) considered the Dictyotales to represent an independent evolutionary lineage, not closely related to the other orders and this was by virtue of Dictyotales' unique uniflagellate spermatozoids and non-flagellate meiosporangia that differ from the more typical unilocular sporangia of other brown algae. Early phycologists even separated it from the Phaeophyceae because of their tetraspores, which resembles those of red algae (De Reviers and Rousseau, 1999). Van den Hoek et al. (1995) regarded Ectocarpales *sensu stricto* as the most primitive of the brown algae on account of their simple filamentous construction while the Fucales and Durvillaeales were considered the most advanced based on the lack of a free-living gametophytic phase.

In subsequent studies, the notion of subdividing the brown algae in classes and subclasses was generally abandoned with only one class recognised. Since the time of Oltmanns (1922), brown algal classification at the ordinal level considers four features of importance: the pattern of life history (isomorphic versus heteromorphic), type of sexual reproduction (iso-, aniso- or oogamy), mode of growth (terminal or intercalary, localised or diffused) and the construction of thallus (pseudoparenchymatous, parenchymatous and filamentous). The Ectocarpales were considered to be the earliest diverging brown algal lineage because they displayed all three character states (De Reviers and Rousseau, 1999). An ectocarpalean ancestor that developed a digenetic haplodiplontic life cycle was believed to give rise to all other lineages except the Fucales. An isomorphic life history together with isogamous fertilisation and haplostichous architecture has been regarded as a *priori* of the most primitive states (Silberfeld et al., 2010).

The current standard for taxonomic and phylogenetic studies adopts both morphology-based and molecular approaches to give a better understanding of the subject of matter (Draisma, 2002). With the advent of molecular data especially DNA sequencing, a multitude of phylogenetic studies have cropped up over the past two decades to infer the phylogeny and evolution of the brown algae. Nevertheless, a universal problem faced by researchers alike remains to be the approach to taxon sampling whereby the chosen representative taxa from different orders varies among workers. This combined with incomplete sequences and/or sequences from different regions of a certain marker or even sequences from different markers resulted in a mosaic view of the Phaeophyceae in which sister-group relationships have remained unsolved (Draisma, 2002). Although life history patterns are still frequently used as a crtiterion in distinguishing the phaeophycean orders, this information is still lacking for a substantial number of taxa (Wynne and Loiseaux, 1976). Undoubtedly, determination of ploidy levels for various stages is critical in unravelling a life history. However, a major hurdle lies in the constant lack of cytological evidence to verify the interpretation of an alternation of generations observed at the morphological level (Wynne and Loiseaux, 1976).

2.2.2 Types of early development of the thallus as a taxonomic criterion

Three types of early development of the thallus are recognised. The creeping-type, exhibited by members of the Ectocarpaceae and Myrionemataceae involves creeping filament that develops first, extends in the horizontal plane and serves as the primary means of attachment to the substratum. The creeping system can be a loosely branched filament, or a richly branched expanse or a compact disc with numerous threads coalescent to form one-layered stratum. The erect system then develops by outgrowth of

cells of the creeping system in a plane perpendicular to its direction of expansion. In the erect-type, there is no prostrate system and growth of the thallus is erect from the beginning and this is exemplified by the Dictyotales and Fucales. Another type of development of the thallus is the discal-type which is one of the criteria for members of Ralfsiales. It has also been described by Kuckuck (1912) in *Nemoderma tingitanum* Schousboe ex Bornet, by Kylin (1934) and Loiseaux (1968) in *Ralfsia clavata* (Carmichael ex Harvey) Crouan & Crouan and by Sauvageau (1907) in members of the Sphacelariales. The swarmers germinate by successive transverse and longitudinal cell divisions into a minute parenchymatous disc from which the erect system arises. Nakamura (1972) noted that forms with this type of discal development are without pyrenoids.

2.2.3 Resolution of brown algal evolutionary relationships through molecular phylogeny

Comparative morphology is traditionally used in the identification of taxa by means of selecting diagnostic characters and deciding upon the degree of morphological variation allowed in a taxon (Draisma, 2002). This approach depends heavily on reliable diagnostic characters and this becomes a drawback if the characters are not found. New specimens were compared against the standard, i.e. the holotype, and assigned accordingly. When sufficient discontinuities were found, a new species and/or new circumscription were made based on taxonomic expertise and judgement. Elsewhere, phylogenetics aims to unravel the shared evolutionary history among taxa by assessing the homology in terms of structure or position. Structural comparisons are common in the case of morphological characters whereas homology is assessed by position in the case of DNA sequence data. The key to phylogenetics is to locate homologous

characters that define monophyletic groups and thus reflect shared common ancestry. The catch here is to find synapomorphies i.e. shared homologous characters that define monophyletic groups instead of 1) the autapomorphies which are characters unique to a single species such that each taxon becomes its own group and deprived us the knowledge of the relationships among the groups and 2) a character that is present in all species as there will be no discriminatory power. Nevertheless, the issue of convergence or homoplasy has to be dealt with and this forms the major shortcoming of phenetic approaches because homologous and convergent characters tend to mix. Through the application of DNA data, the number of characters is limited only by the availability of sequences having sufficient variation for the level of the question being addressed (Draisma, 2002).

Insights into the taxonomy and evolutionary relationships within the Phaeophyceae have been steadily emerging since the early 1990s through the contribution of molecular data. Pioneer studies were rather limited in scope and do not feature a combination of strong taxon and gene sampling which is important in resolving rapid diversifications. These limitations were gradually circumvented with increasingly comprehensive taxon and gene sampling (e.g., Bittner et al., 2008; Silberfeld et al., 2010). Some of the main defining molecular studies leading to modification of classification at the ordinal level, or to new phylogenetic hypotheses are presented here. The concept of Ectocarpales *sensu lato* being proposed to include the Scytosiphonales, Chordariales and Dictyosiphonales to represent a well-supported monophyletic group was one of the earliest changes proposed (Tan and Druehl, 1993). The concept of the presence of pyrenoids and/or number and ultrastructure of chloroplasts was adopted by a number of researchers (Kawai, 1991; Rousseau and De Reviers, 1999). Multiple plastids without pyrenoids appear to be the plesiomorphic (an ancestral trait) condition in the brown algae. Rousseau and De Reviers (1999) regarded

stalked pyrenoids as a synapomorphy (a derived trait that is shared by two or more species or groups) of the Ectocarpales which can be used to define the order. They proposed a new delineation for Ectocarpales *sensu lato* to include Ectocarpales *sensu stricto*, Chordariales, Dictyosiphonales, Punctariales and Scytosiphonales, all of which possess one or several plastids, each with one or several stalked pyrenoids. The Tilopteridaceae, Ralfsiales, Scytothamnales, *Asteronema*, *Bachelotia* and *Asterocladon* which have either plastids without pyrenoids or non-pedunculate pyrenoids were removed from the Ectocarpales.

Subsequent studies focused on a lower taxonomic level for e.g., circumscribing limits of the Ectocarpales (Rousseau and De Reviers, 1999) and unravelling the relationship within the Fucales (Cho et al., 2006). In 2001, Rousseau et al. produced the first comprehensive phylogeny of the Phaeophyceae using partial SSU and LSU rDNA sequences. Their results showed several important findings: the Fucales are not sister to the rest of the brown algae, the Dictyotales instead of the Ectocarpales make an early divergence, apical growth and polystichous thallus structure are ancestral and not derived as previously thought, and *Nemoderma* appears to be sister to the Fucales. Draisma et al. (2001) produced the same result with the additional findings of *Choristocarpus* (not included in the study by Rousseau et al. 2001) being sister to the rest of the brown algae. This piece of information revolutionised the brown algal phylogeny in which isomorphic life cycle and apical growth are plesiomorphic in the brown algae, contrary to all previous phylogenetic hypotheses. Kawai et al. (2007) who based their study on *rbc*L and partial 18S rDNA sequences reported that together with *Choristocarpus*, the monotypic genus *Discosporangium* forms a monophyletic clade that is sister to all other brown algae and this result holds this present. The order Discosporangiales was reinstated to accommodate them. A study by Cho et al. (2004) using the three plastid-encoded genes (*rbc*L, *psa*A, *psb*A) lead to the creation of the

order Ishigeales on the basis of the genus *Ishige* which was previously included in the Ectocarpales *sensu lato*. This order diverges early after the *Choristocarpus* and the *Ishige* which lacks pyrenoids are consistent with the new delineation of Ectocarpales by Rousseau and De Reviers (1999). In a study by Silberfeld et al. (2011) on the systematics and evolutionary history of pyrenoid-bearing taxa in the brown algae, a new order, Asterocladales was established to accommodate species of the genus *Asterocladon*.

The nuclear ribosomal DNA (rDNA) small subunit gene (18S) is regularly used by most researchers but the resolving power of the 18S alone has been proven to be insufficient in dealing with the relative recency of the various brown algal lineages (Draisma et al., 2001). Likewise, results obtained with partial sequences of the rDNA large subunit gene did not give better resolution (Rousseau et al., 2000). Rapidly evolving sequences such as the internal transcribed spacer (ITS) region are more suited for construction of phylogenies at the species level whereas genes that are more conserved, for e.g., *rbc*L are better suited for phylogenetic studies at the familial or ordinal level. The ITS regions are relatively free of evolutionary constraints since they do not code for any particular function unlike protein-coding genes such as *rbc*L and thus they show considerable length variation. In view of the hundreds to thousands of repeats of rDNA cistron in typical nuclear genomes, the ITS regions are more easily amplified than most low-copy nuclear loci (Alvarez and Wendel, 2003). However, the use of the ITS region may be discouraged by its difficulty in alignment and presence of pseudogenes with different base compositions as opposed to protein-coding genes which do not face this problem (Cho et al., 2009). Draisma et al. (2001) observed that *rbc*L provides better resolution than rDNA in unravelling the phylogenetic relationships of the Phaeophyceae although both genes were highly conserved. The *psa*A gene (from Cho et al., 2004) has also been proven to be much more informative at the class level.

Some of the recent phylogenetic studies used a combination of markers which include the mitochondrial-encoded *cox*1 gene, a marker which has also been proposed for barcoding (Bittner et al., 2008; McDevit and Saunders, 2009; Silberfeld et al., 2010).

Despite the many advantages of using DNA sequence data in comparison to the phenetic and morphology-based approach, we must be aware of the possibility that DNA sequence data are being published under the wrong taxonomic names. This can be due to misidentification of the studied specimen or the contamination by DNA of another organism during PCR amplification (De Reviers et al., 2007). Apart from that, most authors have found it difficult to compare their own results to previously published studies as they were faced with differences in taxon sampling, use of different genes, use of partial sequences (but then from different regions of the same gene in different studies), choice of outgroup taxa and different analysis methods (Draisma et al., 2001).

The future direction to be taken involves the identification of suitable universal marker(s) for barcoding purpose which will prove extremely useful for notoriously difficult taxa such as species of *Sargassum.* Highly variable markers will be required for this routine and studies have shown their potential to reveal cryptic species within a (putative) morphological species (Saunders, 2008; Freshwater et al., 2010). Genome sequencing may open the floodgate to the identification of new markers and design of further probes. Recent studies seemed to imply that inserted sequences like the intron observed in the mitochondrial genome of some brown algae are promising as phylogenetic reconstruction tools (Oudot-Le Secq et al., 2006). Thus, comparison of complete genomes may uncover other introns, transposable element families, repeated elements or any rare genetic event that may be useful for phylogenetic investigations.

Several approaches can be used in phylogeny reconstruction and these include neighbour joining (NJ), maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI). Soltis and Soltis (2003; Table II, page 1797) summarised some

of the strengths and weaknesses of these approaches which depend on the type, quality and quantity of data as well as the size of the group under study, and is reproduced here with modification as Table 2.1.

Table 2.1 Summary of various methods of phylogenetic analyses (Adapted from Soltis and Soltis, 2003 with modification).

Method	General	Advantages	Disadvantages
Parsimony	Select the tree or trees that minimize the amount of change (no. of steps)	By minimizing no. of steps, it also minimizes the no. of additional hypothesis	Different results may be obtained based on the entry order of sequences (therefore perform multiple searches)
		Searches identify numerous equally parsimonius (shortest) trees; treats multiple hits as an inevitable source of false similarity (homoplasy)	Relatively slow (compared with NJ) with large data sets
		Readily implemented in PAUP*	Highly unequal rates of base substitution may cause difficulties (e.g. long branch attraction)
		Can identify individual characters that are informative or problematic	
		Can infer ancestral states	
Neighbour joining (NJ)	Involves estimation of pair-wise distances between nucleotide sequences	Fast	Different results may be obtained based on the entry order of sequences
	Pair-wise distances compensate for multiple hits by transforming observed percent differences into an estimate of the no. of nucleotides substitutions using one of several models of molecular evolution	Provides branch lengths	Only a single tree produced; cannot evaluate other trees
	Minimum evolution is a common distance criterion for picking an optional tree (sum of all branch lengths is the smallest)	Uses molecular evolution model	Branch lengths presented as distances rather than as discrete characters (steps)
	NJ algorithm provides a good approximation of the minimum evolution trees	Readily implemented in PAUP* and MEGA	Cannot identify characters that are either informative or problematic
			Cannot infer ancestral states

Table 2.1 (continued)

Method	General	Advantages	Disadvantages
Maximum Likelihood (ML)		A statistical test (the likelihood ratio test) can be used to evaluate properties of trees	Computationally very intensive (much slower than other methods)
		Nucleotide substitution models are used directly in the estimation process, rather than indirectly (as in parsimony)	Practical with only small nos. (fewer than 50) of sequences
		Flexible, models that can incorporate parameters of base frequencies, substitution rates, and variation in substitution rates and therefore are "general"	
		Readily implemented in PAUP*	
		Uses all of the data (invariable sites and unique mutations are still informative, unlike parsimony analysis)	
Bayesian	Uses a likelihood function and an efficient search strategy	Based on the likelihood function, from which it inherits many of its favourable statistical properties	Very large memory demands
	Based on a quality called the posterior probability of a tree	Uses models as in ML	
	Researcher may specify belief in a prior hypothesis prior to analysis	Can be used to analyse relatively large data sets	
		Provides support values	Posterior probabilities (measure of internal support) can be overestimates

2.3 Crustose brown algae and the controversies concerning their systematic treatment at the ordinal and familial levels

The uncertainty and confusion of the taxonomy and systematics of the crustose brown algae can be seen via their various placings in several orders and families. Most of them were placed in the families Ralfsiaceae and Lithodermataceae (e.g., Hauck, 1885; Børgesen, 1914; Tanaka and Chihara, 1980 a-c; Tanaka and Chihara, 1981 a-c) and it

was not uncommon to find crustose brown algae placed in the Ectocarpales (Feldmann, 1937) or the Chordariales (Agardh, 1848 as Chordarieæ) before the Ralfsiales was established. Nevertheless, Abbott and Hollenberg (1976) and Womersley (1987) retained the Ralfsiaceae in Chordariales even after the establishment of Ralfsiales while Bold and Wynne (1985) and Tanaka and Chihara (1980 - 1982) adopted the use of Ralfsiales. At the familial level, a number of workers recognised both the Ralfsiaceae and Lithodermataceae (e.g., Taylor, 1957; Bold and Wynne, 1985) while others acknowledge only the Ralfsiaceae (e.g., Hollenberg, 1969; John and Lawson, 1974). The similarities between the Myrionemataceae and the Ralfsiaceae/ Lithodermataceae have also been highlighted (e.g., Setchell and Gardner, 1925; Fletcher, 1978) with some workers assigning all these algae to one single family, the Myrionemataceae (Skottsberg, 1921; Loiseaux, 1968). Rosenvinge (1893) for instance, placed the genera *Ralfsia* and *Lithoderma* in the Myrionemataceae while Fletcher (1978) placed the crustose brown algae in two families, the Ralfsiaceae and Myrionemataceae. Some examples of their inclusion in the Ectocarpales and Chordariales are provided as follows.

Fritsch (1945) included members of the Ralfsiaceae (*Analipus* and *Ralfsia*), which did not have an ordinal status at that time in the merger of Chordariales, Dictyosiphonales, Ectocarpales and Scytosiphonales into the Ectocarpales *sensu lato*. The family Ralfsiaceae was grouped under Ectocarpales along with the family Ectocarpaceae by Papenfuss (1951a). The following genera were included in his family of Ralfsiaceae: *Ralfsia* Berkeley (including *Stragularia* Strömfelt), *Mesospora* Weber-van Bosse, *Nemoderma* Schousboe ex Bornet, *Petroderma* Kuckuck, *Hapalospongidion* Saunders, *Heribaudiella* Gomont, *Sorapion* Kuckuck, *Lithoderma* Areschoug (including *Pseudolithoderma* Svedelius), *Hapterophycus* Setchell & Gardner, *Acrospongium* Schiffner, and *Symphyocarpus* Rosenvinge. Meanwhile, Taylor (1957)

followed Agardh's (1848) scheme by including *Ralfsia, Sorapion* and *Lithoderma* in the Chordariales with the last two genera being placed in the Lithodermataceae.

A number of researchers preferred to place certain members of the crustose brown algae in the family Scytosiphonaceae. For instance, Pedersen (1976) placed *Ralfsia* in the Scytosiphonaceae of the Scytosiphonales with Myrionemataceae and Lithodermataceae in the Ectocarpales while Christensen (1980) assigned *Ralfsia* and *Analipus* to the Scytosiphonaceae. Several crustose brown algae have been identified as stages of taxa with heteromorphic life cycles, particularly species of the Scytosiphonaceae. *Ralfsia clavata* and *Microspongium gelatinosum* Reinke were shown to be crustose phases in the life histories of *Petalonia fascia* (O.F.Müller) Kuntze and *Scytosiphon lomentaria* (Lyngbye) Link, respectively (Fletcher, 1974).

Some workers consider *Ralfsia* crusts involved in scytosiphonalean life histories to be under the subgenus *Stragularia* (e.g., Wynne, 1969; Edelstein et al., 1970). *Stragularia*, initially described as a genus by Strömfelt (1886) was later given subgeneric rank with *Euralfsia* by Batters (1890) who distinguished *Stragularia* from *Euralfsia* by having a looser vegetative structure and diffuse sporangia.

2.4 Ralfsiales Nakamura ex Lim & Kawai

Nakamura established the order Ralfsiales in 1972 on the basis of the genus *Ralfsia* to accommodate the crustose brown algal taxa (with the exception of *Analipus*). Members of this order are characterised by having isomorphic life history with alternating haploid and diploid phases (=diplohaplontic) in which both sporophytes and gametophytes consist of haplostichous tissues (pseudoparenchymatous thalli construction); discal-type early development of thalli; type of sexuality in the form of a direct apomeiotic cycle and cells with a single, parietal, plate-like chloroplast without pyrenoid. *Hapterophycus*

canaliculatus Setchell & Gardner which exhibits the creeping-type of development of thalli and the *Scytosiphon*-type life cycle was removed from the Ralfsiaceae to the Scytosiphonaceae by Nakamura (1972). A study by Kogame (1996) found the Japanese specimens of *H. canaliculatus* (generitype of *Hapterophycus*) to be synonymous with *Scytosiphon canaliculatus* (Setchell & Gardner) Kogame, thus reducing *Hapterophycus* to a synonym of *Scytosiphon* (Scytosiphonaceae). However, Wynne (1969) did not observe erect thalli in culture studies of *H. canaliculatus* from California. The generic and familial status of *Hapterophycus* will need to be resolved by molecular and phylogenetic analyses (Norris, 2010).

Three families i.e. Lithodermataceae Kjellman, Nemodermataceae Feldmann and Ralfsiaceae Farlow were initially included in Ralfsiales by Nakamura and the distinction among them was mainly based on their reproductive structures (Fig. 2.1). Lithodermataceae has both the unilocular and plurilocular sporangia located terminally. For Nemodermataceae, the unilocular sporangia are intercalary and the plurilocular sporangia are laterally inserted while the Ralfsiaceae possess lateral unilocular sporangia and intercalary plurilocular sporangia. Nakamura (1972) also implied the possibility of merging them into a single family, the Ralfsiaceae. The main distinguishing features of Ralfsiales were (1) isomorphic life history; (2) a single, parietal, plate-shaped chloroplast without pyrenoids in each cell; and (3) discoid early development of the thallus. The order was not recognised as a valid publication because no Latin description was provided by Nakamura (1972), thus the name failed to comply with the requirement of the International Code of Botanical Nomenclature (see McNeill et al., 2006: Art. 36.2; currently known as the International Code of Nomenclature for algae, fungi and plants). In addition, several exceptions to the distinguishing features mentioned above have been found within the order. In spite of this, Silva and De

Reviers (2000) noted that, the name, although not validly published, had been used in the literature by many phycologists.

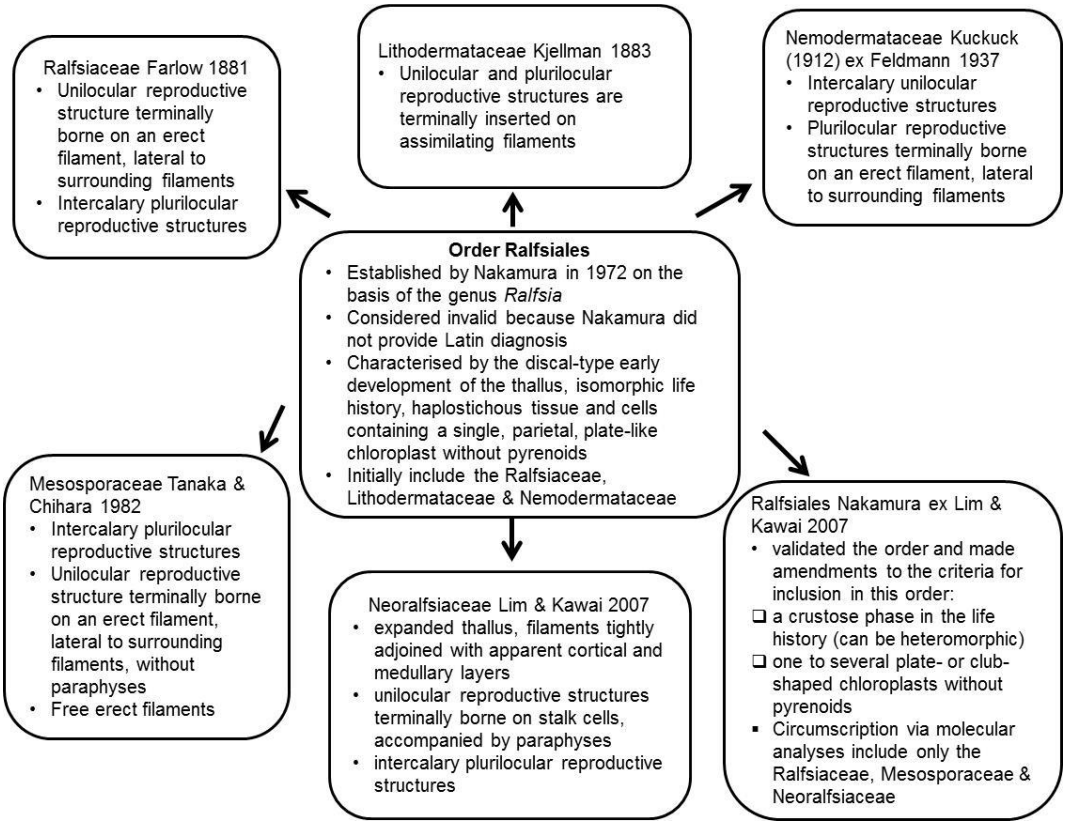

Figure 2.1 A schematic diagram of the families included in the order Ralfsiales.

Some researchers disagreed with the placement of crustose brown algae under the Ralfsiales (John and Lawson, 1974; Russell and Fletcher, 1975; Abbott and Hollenberg, 1976). Nelson (1982) recommended the discontinuation of the Ralfsiales owing to the "inconsistencies in the delimitation of the Ralfsiales" and proposed that all crustose brown algae be placed under a single family, the Ralfsiaceae under Ectocarpales. She placed both *Ralfsia fungiformis* (Gunnerus) Setchell & Gardner and *Analipus japonicus* (Harvey) Wynne under Ectocarpales. The order Ralfsiales was accepted by various authorities prior to and since Nelson's recommendation (Tanaka and Chihara, 1982; Pritchard and Bradt, 1984; Bold and Wynne, 1985) while some

continued not to recognise it (Gabrielson et al., 1989). Tanaka and Chihara (1980a) accepted the Ralfsiales but disagree with the use of number of chloroplasts per cell as a criterion for this order as they mentioned that there are some inconsistencies in the number and shape of chloroplast for some species. In 1982, they proposed an establishment of a new family, Mesosporaceae in the Ralfsiales which comprise three genera: *Mesospora*, *Hapalospongidion* and *Basispora*. Kawai (1989) showed the close systematic position of *Gobia saxicola* Okamura & Yamada, which was originally described as a chordarialean species, with ralfsiacean crustose algae, and transferred it to Ralfsiales. Therefore, the order was shown to include a taxon showing a heteromorphic life history. The first molecular study by Tan and Druel (1994) on *R. fungiformis* and *A. japonicus* using 18S rDNA showed that these two species should not be placed in Ectocarpales but no further conclusion was made on their systematic position. Structurally, *Ralfsia* is compactly arranged, with pseudoparenchymatous aggregations of filaments into prostrate crusts while *Analipus* has erect, branched axes from a prostrate crust.

Lim and Kawai were the first to validate the ordinal name of Ralfsiales in 2007 with Latin diagnosis, based on morphological features and *rbc*L gene sequences (Lim et al., 2007). The characteristic features of Ralfsiales laid out by these authors were somewhat different from what Nakamura proposed in 1972. The revised features of Ralfsiales include (1) discoid early development of the thallus; (2) one to several plate- or club- shaped chloroplasts without pyrenoids; (3) plurilocular zoidangia with sterile terminal cell(s) and terminal unilocular zoidangia; and (4) presence of crustose gametophytic or sporophytic stage(s) in the life history (see Figs. 2.2 - 2.5). They established a new family, Neoralfsiaceae, to accommodate the new genus *Neoralfsia*, based on *Ralfsia expansa* (J. Agardh) J. Agardh. With the new amendment, Ralfsiales consists of only the Ralfsiaceae, Neoralfsiaceae and Mesosporaceae. The

Nemodermataceae and Lithodermataceae were not included in this order but further conclusion on their phylogenetic position can only be determined by conducting molecular analyses on authentic specimens of these families, thus marking a difference from the description by Nakamura (1972).

Members of Ralfsiales have very simple morphological features (mostly crusts tightly attached on rocks), therefore limiting the taxonomic characters based on morphology and anatomy as compared to other taxa. Traditionally the identification up to genus and species level is based on the construction of thalli, life history patterns, occurrence of sessile or pedicellate unilocular sporangia associated with multicellular paraphyses and plurilocular sporangia with sterile terminal cells. In spite of all the above mentioned criteria, the identification of crustose brown algae is challenging because mature thalli with reproductive structures are seasonal and elusive in the field. A direct type of development is typically reported for members of this order. In agreement with Nelson (1982), chloroplast-related traits which include the number, shape and the presence or absence of pyrenoids are reported with considerable variation among the crustose brown algae.

Through the advent of molecular tools, confusions or contradictions that arose in the past regarding the validity of the Ralfsiales (e.g., Nelson, 1982) and the families recognised under Ralfsiales (e.g., Fletcher, 1978) can now be properly resolved and better understood. Members of the Lithodermataceae such as *Pseudolithoderma roscoffense* Loiseaux have been demonstrated to be distantly related to members of the Ralfsiales (Bittner et al., 2008). On the other hand, *Nemoderma tingitanum*, the sole member of Nemodermataceae was accorded its own order, the Nemodermatales (Phillips et al., 2008).

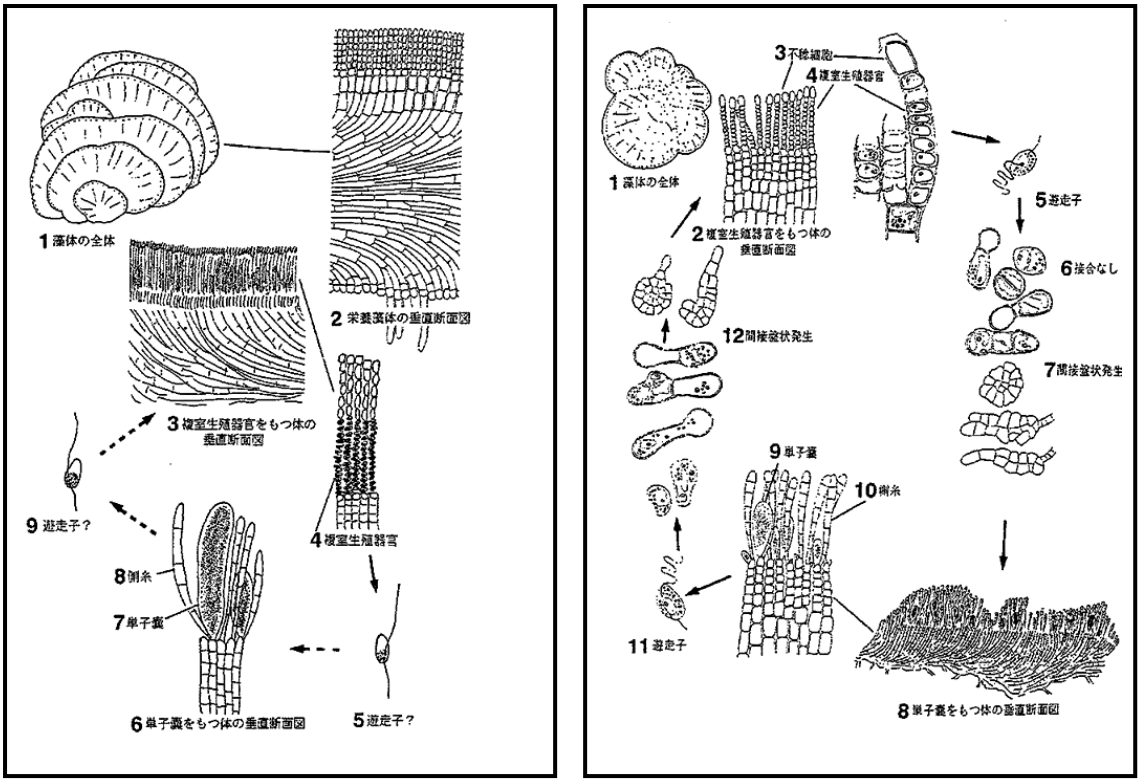

Figure 2.2 Life histories of *Ralfsia fungiformis* (Gunnerus) Setchell & Gardner (left) and *R. verrucosa* (Areschoug) Areschoug (right) [after J. Tanaka in Hori 1993, pp. 50 - 53, figs. 25 - 26]. For *R. fungiformis*: 1. Natural habit of thallus; 2. cross section of a vegetative thallus; 3. cross section of a thallus with plurilocular reproductive structures; 4. plurilocular reproductive structures; 5. swarmer?; 6. cross section of a thallus with unilocular sporangia; 7. unilocular sporangia; 8. paraphysis; 9. swarmer?. For *R. verrucosa*: 1. Natural habit of a thallus; 2. cross section of a thallus with plurilocular reproductive structures; 3. terminal sterile cells; 4. plurilocular reproductive structures; 5. swarmer; 6. parthenogenesis; 7. indirect type of germination to form a disc; 8. cross section of a thallus with unilocular sporangia; 9. unilocular sporangium; 10. paraphysis; 11. swarmer; 12. indirect type of germination to form a disc.

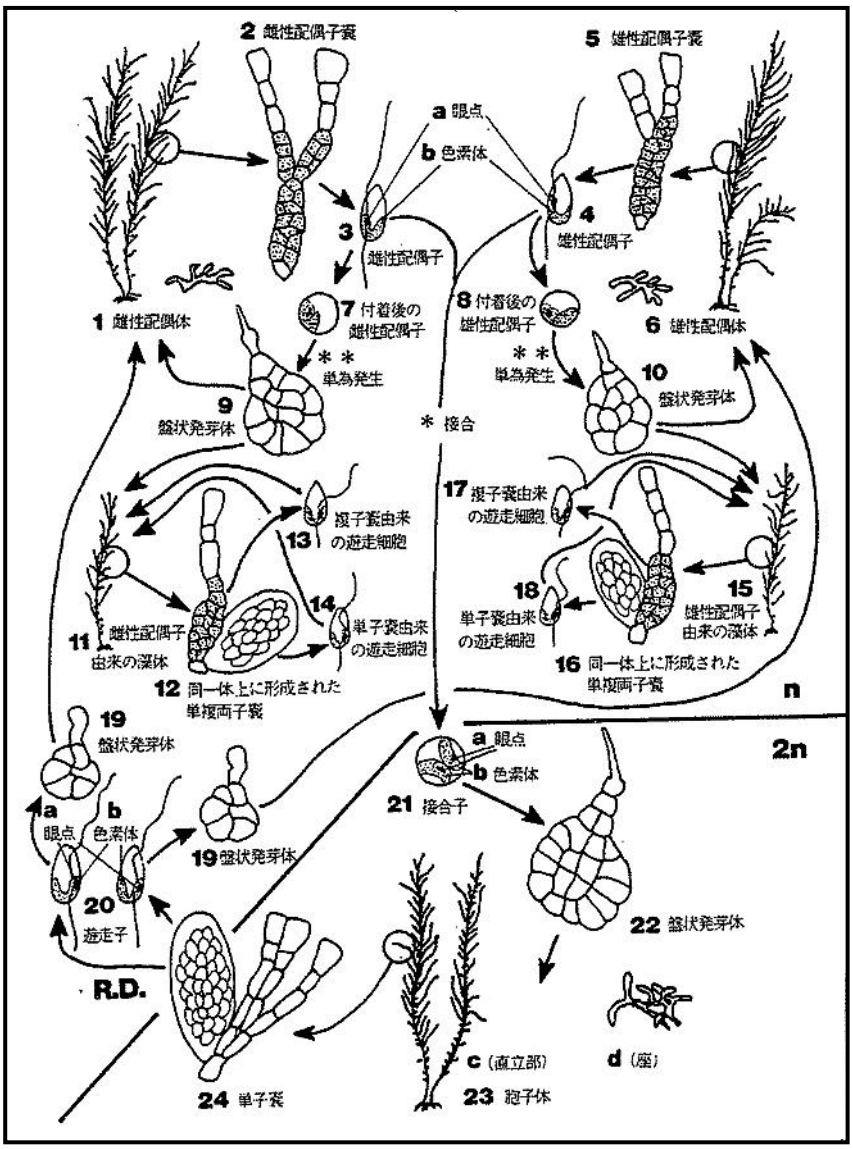

Figure 2.3 Life history of *Analipus japonicus* (Harvey) Wynne (after H. Nakahara in Hori 1993, pp. 42 - 43, fig. 21). 1. Female gametophyte; 2. female gametangium; 3. female gamete; 4. male gamete; 5. male gametangium; 6. male gametophyte; 7. settled female gamete; 8. settled male gamete; 9, 10, 19, 22. discoid germlings; 11. erect frond from female gamete; 12, 16. uni- and plurilocular sporangia on the same frond; 13, 17. pluriswarmer; 14, 18. uniswarmer; 15. erect frond from male gamete; 20. unispores; 21. zygote; 23. sporophyte; 24. unilocular sporangium; a, eyespot. b, chloroplast. c, erect frond (winter form). d, prostrate thallus (summer form) * conjugation of gametes ** parthenogenesis.

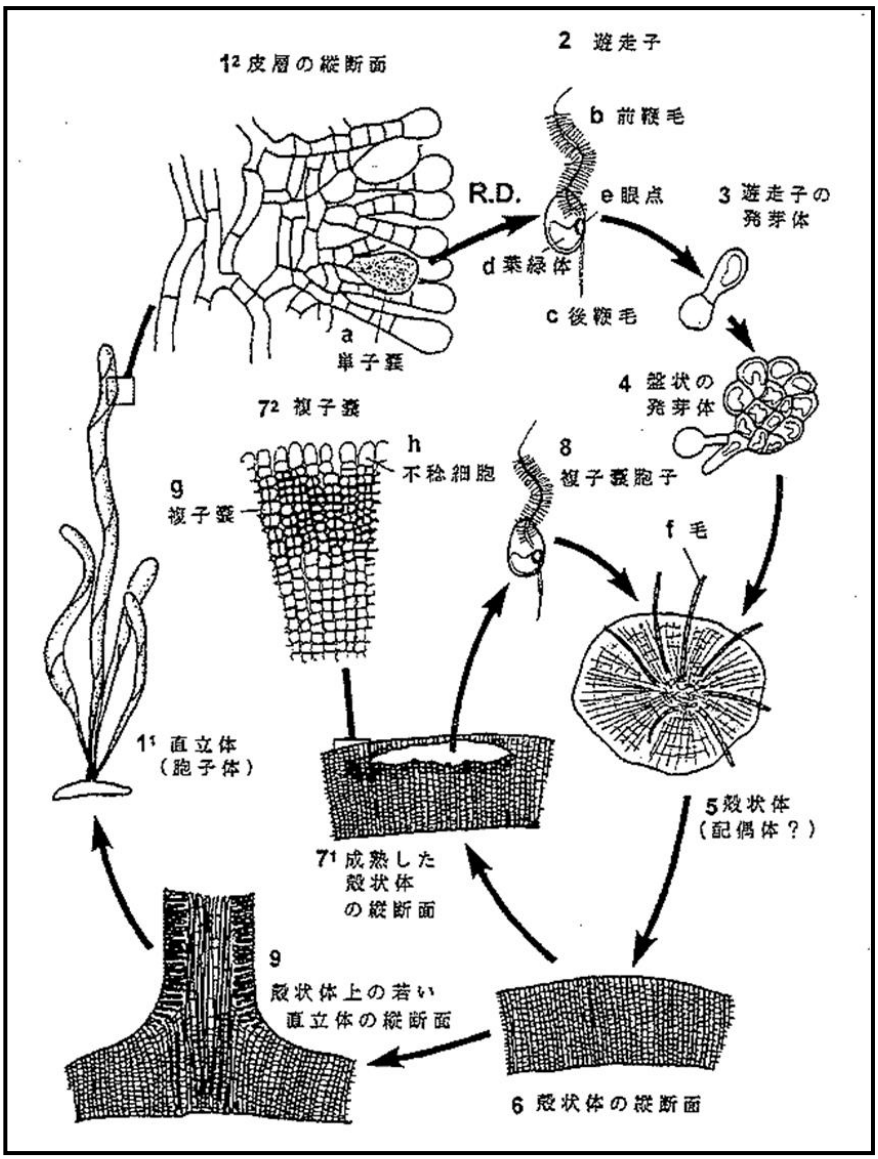

Figure 2.4 Life history of *Heteroralfsia saxicola* (Okamura & Yamada) Kawai (after H. Kawai in Hori 1993, pp. 46 - 47, fig. 23) 1^1. Habit of erect thallus (sporophyte); 1^2. cortical layer in longitudinal section; 2. zoospore; 3. germination of zoospore forming emptied embryospore; 4. discal germination of zoospore; 5. crustose thallus (putative gametophyte); 6. longitudinal section; 7^1. partly emptied plurilocular sporangia; 7^2. plurilocular sporangia with terminal sterile cells; 8. plurispore (putative gamete); 9. development of erect thallus on crustose thallus. a, unilocular sporangium. b, anterior flagellum. c, posterior flagellum. d, chloroplast. e, eyespot. f, phaeophycean hair. g, plurilocular sporangia. h, sterile cell.

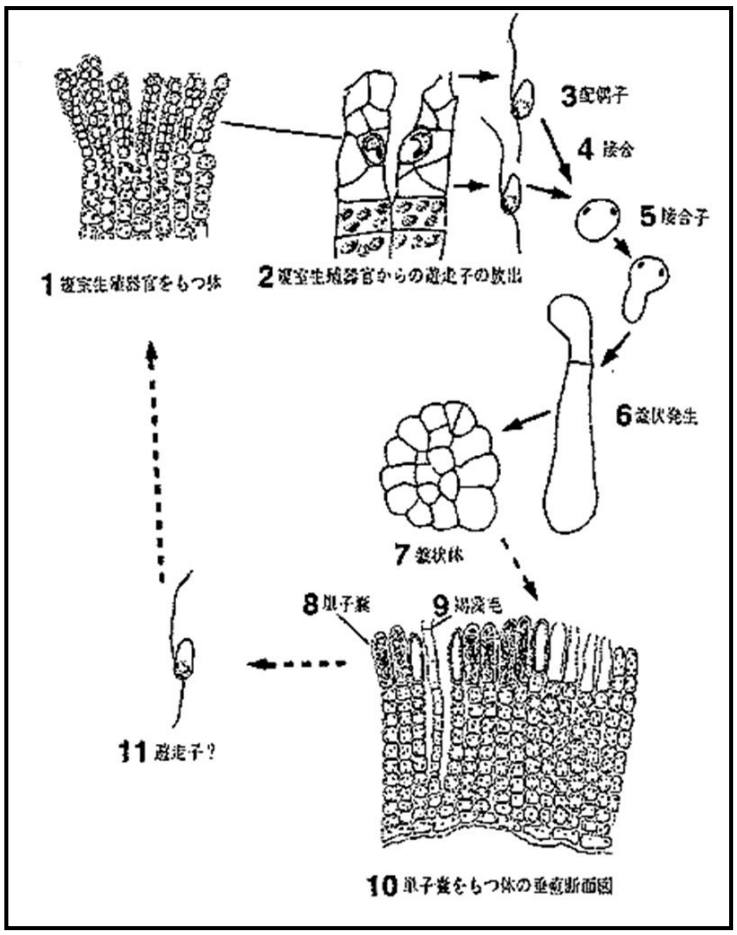

Figure 2.5 Life history of *Pseudolithoderma subextensum* (Waern) Lund (after J. Tanaka in Hori 1993, pp. 48 - 49, fig. 24). 1. Cross section of a thallus with plurilocular reproductive structures; 2. release of gametes from plurilocular reproductive structures; 3. gametes; 4. copulation; 5. zygote; 6. germination to form a crust; 7. disc; 8. unilocular sporangia; 9. phaeophyceaen hair; 10. cross section of a thallus with unilocular sporangia; 11. swarmer (2 - 7, as *P. extensum*, after Peters 1989)

2.4.1 Ralfsiaceae Farlow

The diagnosis of this family as proposed by Farlow (1881, as Ralfsieæ) includes a thallus in the form of a crust, fruiting in superficial sori comprising spheroidal unilocular sporangia and club-shaped paraphyses. Subsequently, many authors gave a more detailed description for this family which include among others, prostrate,

rounded to irregularly spreading thallus firmly adherent or easily detached on the substratum, with or without rhizoids on the ventral surface; basal plate one to several cells thick from which erect filaments, closely adherent, generally unbranched or sparsely branched, with unilateral or bilateral disposition, arise; chloroplasts one to several in each cell, without pyrenoids and intercalary plurilocular sporangia.

The life history for members of this family is presumably diplohaplontic and isomorphic, but probably more frquently direct from plurilocular sporangia or apomeiotic unilocular sporangia. The sporophytes often reproduce by apomeiotic unilocular meiosporangia and the gametophyte by plurilocular reproductive structures with neutral spores (Womersley, 1987). Fletcher's (1978) description of this family stated that members are predominantly characterised by a discoid base giving rise to laterally coherent vegetative filaments and the undifferentiated nature of the vegetative filaments in the crusts set them apart from the more elaborate pustules and cushions of algae in the families Elachistaceae and Corynophlaeaceae (both currently placed in the Chordariaceae, Ectocarpales *sensu lato*).

2.4.1.1 *Ralfsia* Berkeley in Smith & Sowerby (Fig. 2.6)

Holotype: *Ralfsia deusta* (C. Agardh) Berkeley

Taxonomic synonym and currently accepted name for type species: *Ralfsia fungiformis* (Gunnerus) Setchell & Gardner

Type locality: Iceland (as *Zonaria deusta* C. Agardh)

Distribution: Widespread on cold temperate to subtropical coasts, probably throughout the world, more so in the northern hemisphere. E.g., Europe: Kuckuck 1894, Hamel 1935, Kylin 1947, Jaasund 1965, Fletcher 1978; North America: Hollenberg 1969, Norris 2010; Asia: Perestenko 1980, Tanaka and Chihara 1980b-c, 1981a; Silva et al.

1987, Kaehler 1994, 1998; Zeng 2009, Van Nguyen et al. 2013; Africa: Ribera et al. 1992; Australia and New Zealand: Womersley 1987, Harper et al. 2012.

Named after an English botanist (John Ralfs), this is the most speciose genus among the crustose brown algae with 19 taxonomically accepted species listed in Algaebase (Guiry and Guiry, 2013). Members of this genus are primarily characterised by forming flat coriaceous expansions comprising closely packed vegetative filaments (which differs from *Myrionema*), not readily separating under pressure, so as to form a pseudoparenchymatous structure; cells containing a single chloroplast without pyrenoids; reproductive structures united in sori, consisting of unilocular sporangia laterally inserted at the base of clavate and loosely associated multicellular paraphyses or plurilocular sporangia borne near the apex of vegetative filaments, uni- to multiseriate, terminated by sterile cell(s); phaeophycean hairs and rhizoids may be present. Plurilocular sporangia were unknown in the initial diagnosis by Berkeley (in Smith 1843) and later in Farlow (1881). Batters (1890) was among the first to report the rare occurrence of plurilocular sporangia in *R. verrucosa* which are formed from the erect filaments of the thallus and unaccompanied by paraphyses. Characters related to the reproductive structures such as number of stalk cells (unilocular sporangia) and number of sterile terminal cells (plurilocular sporangia) are mainly used to distinguish species of *Ralfsia* (Tanaka and Chihara, 1980a).

Ralfsia has been associated with two sub-genera, *Euralfsia* Batters and *Stragularia* Strömfelt. Ralfsioid crusts involved in scytosiphonalean life histories were sometimes grouped in the subgenus *Stragularia* which was first decribed as a genus by Strömfelt (1886) and later given subgeneric rank with *Euralfsia* by Batters (1890). *Stragularia* is distinguished from *Euralfsia* by having a looser vegetative structure, straight (not curved) vegetative filaments and diffuse sporangia. Batters (1890, 1902)

included *R. verrucosa* in the subgenus *Euralfsia* while *R. spongiocarpa* Batters, *R. clavata*, *R. pusilla* (Strömfelt) Foslie and *R. disciformis* Crouan & Crouan were placed under the subgenus *Stragularia*. Taxonomic uncertainty between both genera has not been properly addressed, for instance *Stragularia pusilla* Strömfelt is currently treated as a taxonomic synonym of *R. pusilla* while *R. spongiocarpa* was transferred to *Stragularia* by Hamel (1939). Currently, two species are recognised under the genus *Stragularia* i.e. the generitype *S. clavata* (Harvey) Hamel and *S. spongiocarpa*. To my knowledge, there is still no clear consensus on the distinction between the genera *Ralfsia* and *Stragularia*, and the use of gene sequencing may solve this complex situation.

The type species, *R. fungiformis* (Fig. 2.6a) is identified by its thallus with imbricated lobes and bilateral symmetry in which vegetative filaments curved upwards and downwards from a medial plane; clear demarcation between cortical and medullary layers; sessile unilocular sporangia borne lateral-basal to the paraphyses and uniseriate plurilocular sporangia terminated by three to six sterile cells. *Ralfsia verrucosa* (Fig. 2.6b) is generally identified by its leathery and verrucose crusts which comprise unilaterally structured vegetative filaments arising curvedly from the basal disc; sessile unilocular sporangia inserted laterally at the base of clavate paraphyses and uniseriate (rarely biseriate) plurilocular sporangia terminated by a sterile cell; both types of reproductive structures are found on separate plants.

Batters (1890) described *R. spongiocarpa* (Fig. 2.6n) with small and thin crusts; cylindrical paraphyses; unilocular sporangia which may be lateral at the base of paraphyses or terminal on the erect filaments; and structures resembling plurilocular sporangia. Fletcher (1978) reported the production of terminal unilocular sporangia unaccompanied by paraphyses in cultures of *R. spongiocarpa*. Furthermore, the same author noted that *R. clavata* and *R. spongiocarpa* have less tightly bound erect filaments

and a monostromatic base compared to *R. verrucosa* and *R. fungiformis* which possess tightly bound erect filaments curving upwards from a multi-layered base.

R. clavata (Fig. 2.6l) is characterised by its small thalli comprising straight erect filaments (not arched as in *R. verrucosa*); unilocular sporangia emerging at the base of club-shaped paraphyses which consist of three to six cells; and multiseriate plurilocular sporangia without sterile terminal cells. Kuckuck (1894) reported that both types of sporangia can be present in the same sorus. Farlow (1881) described *R. clavata* as "a *Ralfsia* with diffuse fructification and slightly developed frond, or a *Myrionema* with an excessively developed basal portion." In 1939, Hamel transferred *R. clavata* to *Stragularia*. In a study by Fletcher (1974), *R. clavata* was shown to be the crustose phase in the life history of *Petalonia fascia* while *R. spongiocarpa* and *R. verrucosa* were found to possess only a crustose phase in a direct type life history, without the presence of a sexual phase.

Ralfsia bornetii (Fig. 2.6e) Kuckuck was first described from Helgoland with growth and construction of sterile thallus similar to *R. verrucosa*; chloroplasts with pyrenoids; sessile unilocular sporangia inserted laterally at the base of paraphyses and multiseriate plurilocular sporangia arising by conversion of terminal cells of the erect filaments. Jaasund (1965) regarded the extreme long basal cell of the paraphyses and the elongated unilocular sporangia as the most conspicuous feature of this species. Tanaka and Chihara's (1980c) alga of the same name was reported with loosely adjoined vegetative filaments (thus directed to *Stragularia*) and lack of plurilocular sporangia, marking a deviation from the description by Kuckuck (1894). Batters (1902), Fletcher (1987) and John et al. (2004) consider *R. bornetii* to be synonymous to *R. clavata*. Edelstein et al. (1970) found that spores of *R. clavata* and *R. bornetii* give rise to *Petalonia*-like thalli of the Scytosiphonaceae and concluded that both species should be referred to *Stragularia* and considered as a single entity. On the contrary, Loiseaux

(1968) obtained a direct crustose stage from spores of unilocular sporangia of *R. clavata*; Edelstein et al. (1971) explained this to be a result of culture conditions or the type of material used as inoculum.

Another species which has been treated as a synonym of *R. clavata* is *Ralfsia tenuis* (Fig. 2.6j), first described by Kylin (1947) who proposed this as a new name for *R. clavata* Reinke (1889). Kylin (1947) considered *R. clavata* (Carmichael ex Harvey) Crouan & Crouan to be *R. verrucosa* (Areschoug) Areschoug and Kuckuck's (1894) version of *R. verrucosa* to be conspecific with his new alga. Kylin's description included thin crust, unilocular sporangia lateral at the base of paraphyses and multiseriate plurilocular sporangia not terminated by sterile cell. Tanaka and Chihara (1980c) described this species from Japan with loosely adjoined vegetative filaments but plurilocular sporangia were not observed. The Crouan brothers (1867) described *R. disciformis* with disc-formed thallus tightly adherent on substratum and unilocular sporangia accompanied by paraphyses. Hamel (1935) cited from Batters (1896) that *R. disciformis* is distinguished from *R. clavata* by its shorter and less clavate paraphyses which are composed of one to two cells. This species is also currently treated as a synonym of *R. clavata*.

Rosenvinge (1893) described *R. ovata* (Fig. 2.6m) with small ovate unilocular sporangia laterally positioned in the middle of the paraphyses, somewhat bearing a resemblance to *Zeacarpa leiomorpha* Anderson, Simons & Bolton (see section 2.4.6.3); each cell with a single plastid while plurilocular sporangia were unknown. Rosenvinge (1899) suggested that the lateral plurilocular sporangia attributed to *Lithoderma fatiscens* Areschoug may be the plurilocular form of *R. ovata*.

Several species of *Ralfsia* have also been reported from the west coast of North America. *Ralfsia californica* was first described by Setchell and Gardner (1924) to be closely related to *R. clavata* and reported to have clavate unilocular sporangia

positioned at the base of paraphyses while plurilocular sporangia were unknown. Setchell and Gardner (1925) distinguished *R. californica* from *R. clavata* by the former's larger and thicker crusts, longer and narrower unilocular sporangia and slightly smaller cells of the basal portion. Hollenberg (1969) described *R. californica* as having strictly erect filaments from a single basal layer, one chloroplast per cell and sessile unilocular sporangia whereas plurilocular sporangia are borne on larger erect stages. Wynne (1969) reported *R. californica* as a stage in the life cycle of *Petalonia fascia*. Setchell and Gardner (1924) described a second species, *R. hesperia* (Fig. 2.6c) which they considered to be of close affinity to *R. verrucosa* but differed by the former's fleshy, thicker and smaller diameter crust, larger unilocular sporangia and longer paraphyses than the latter. Plurilocular sporangia are unknown in *R. hesperia*. Hollenberg's (1969) description of *R. hesperia* included cell rows directed upward and sometimes also downward, one chromatophore per cell and unilocular sporangia sessile or on short one-celled stalk.

Hollenberg (1969) described two new species from California. *Ralfsia confusa* Hollenberg (Fig. 2.6g) was characterised by having thin crusts; strictly erect filaments; unilocular sporangia sessile or on one to three to six-celled stalks (frequently elongate as successive sporangia are formed on the same stalk) basal-lateral to paraphyses and plurilocular sporangia on separate plants, mostly uniseriate and with a single sterile terminal cell. Hollenberg (1969) treated this species as a member of *Stragularia* on the basis of its thin crust and non-assurgent vegetative filaments. The other species, *R. integra* Hollenberg (Fig. 2.6h) was described with more or less assurgent filaments arising from the basal layer, single plastid per cell, unilocular sporangia sessile at the base of paraphyses or becomingly distinctly stalked as new sporangia proliferates while plurilocular sporangia were unknown. According to Tanaka and Chihara (1980c), this species is separated from other members of *Ralfsia* by having longer cortical cells,

paraphyses and unilocular sporangia. Dawson (1944) described *Ralfsia hancockii*, a species which bears a striking resemblance to another species currently known as *Neoralfsia expansa* (J. Agardh) Lim & Kawai ex Cormaci & Furnari (see section 2.4.5.1 for more details of *R. hancockii*).

Ralfsia occidentalis Hollenberg (in Taylor, 1945) forms a thallus with most cell rows curving upward, plurilocular sporangia terminated by a sterile cell and unilocular sporangia inserted among paraphyses and is thought to be similar to *R. verrucosa* in lacking horizontal stratification. *Ralfsia pacifica* Hollenberg (Fig. 2.6d) is defined by assurgent filaments curving upward, one plastid per cell, unilocular sporangia mostly sessile at the base of paraphyses and uniseriate plurilocular sporangia terminating in a sterile cell. Hollenberg (1969) considers this species to be conspecific with *R. occidentalis* and distinguished *R. pacifica* and *R. verrucosa* based on *R. pacifica*'s regularly uniseriate plurilocular sporangia and non multistratose thallus; however Hollenberg (1969, fig. 17) and Abbott and Hollenberg (1976, fig. 135), illustrated two partly biseriate plurilocular sporangia for *R. pacifica*. Meanwhile, Tanaka and Chihara (1980b) suggested using the size of unilocular sporangia to distinguish these two species in which *R. pacifica* possess longer sporangia than *R. verrucosa*. According to Womersley (1987) who considered *R. pacifica* and *R. verrucosa* to be conspecific, *R. australis* Skottsberg (1921) from the Falkland islands is likewise closely related but differs in lacking hair. *Ralfsia australis* is described with scaly surface, absence of hairs, rarely found and mostly uniseriate plurilocular sporangia while unilocular sporangia were unknown. Skottsberg (1921) also likened his alga to *R. expansa* sensu Børgesen (1912, 1914) and added that unilocular sporangia of *R. australis* are required for better identification.

Lund (1967) described *Ralfsia lucida* with unilocular sporangia which may be terminal, lateral or intercalary in position. *Ralfsia pusilla* is primarily reported to be

epiphytic on other algae or seagrasses (Foslie, 1892; Jaasund, 1965; Fletcher, 1978), possesing loosely connected erect filaments and unilocular sporangia accompanied by paraphyses while plurilocular sporangia were not observed. With reference to the illustrations by Barton (1903, pl. 13), *Ralfsia ceylanica* Harvey ex Barton is probably misidentified as a species of *Ralfsia*. According to Silva et al. (1996), *R. ceylanica* has been referred to *Lobophora variegata* (Lamouroux) Womersley ex Oliveira. *Ralfsia major* Kützing is another species misidentified as a member of *Ralfsia*. Perestenko (1980) described a species of *Ralfsi*a from Russia named *R. longicellularis* (Fig. 2.6k). This species is described with few details including curved ascending filaments and unilocular sporangia borne on multicellular stalks while plurilocular sporangia were not mentioned.

Later, Tanaka and Chihara (1981c) reported two new species from Japan: *R. endopluroides* (Fig. 2.6i) and *R. pedicellata* (Fig. 2.6f). The former is characterised by slightly assurgent filaments firmly adjoined with one another, a single plastid per cell, sessile unilocular sporangia borne at the base of paraphyses and biseriate plurilocular sporangia terminated by three sterile cells (a feature similar to *Endoplura aurea* Hollenberg and thus its specific epithet). Meanwhile, *R. pedicellata* is described with loosely united erect filaments, one chloroplast per cell, unilocular sporangia borne on a small one-celled stalk at the base of paraphyses and biseriate plurilocular sporangia terminated by two sterile cells.

Filamentous development was reported for germlings of *R. integra, R. bornetii* and *R. confusa* (Hollenberg, 1969; Edelstein et al., 1970; Wynne, 1972) while the non-discoidal early development of *R. clavata* is well documented (Nelson, 1982). Nelson (1982) reasoned that the variations shown probably reflect the state of *Ralfsia* taxonomy (heterogeneous composition) rather than the value of this trait at the generic level. In agreement with Buchanan (2005), molecular approach could be used to resolve the

relationships between species of *Ralfsia* and to clarify the circumscription of the Ralfsiaceae and Scytosiphonaceae.

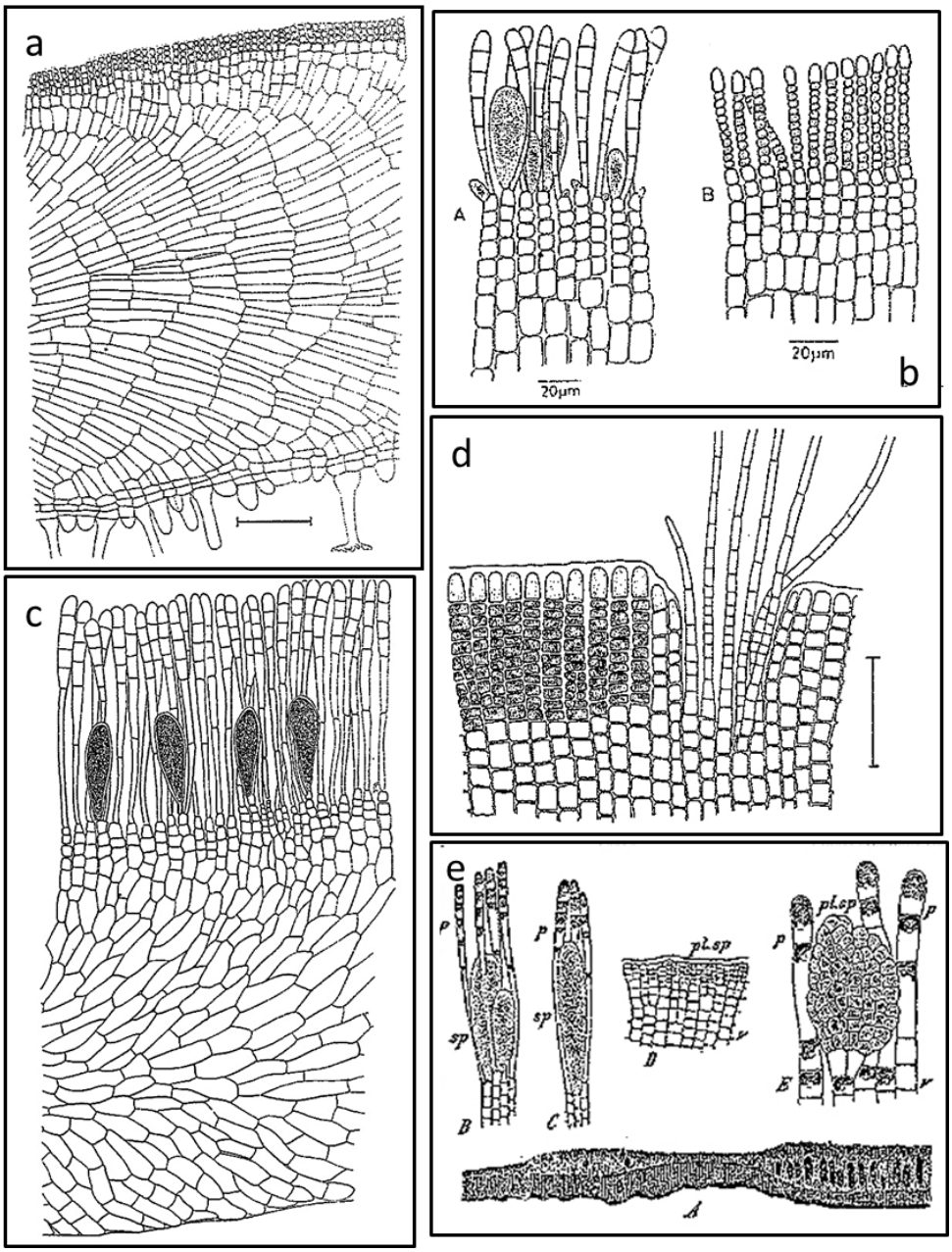

Figure 2.6 *Ralfsia* spp. a. *R. fungiformis* showing vegetative thallus with bilateral symmetry (after Hollenberg 1969, p. 300, fig. 29); b. *R. verrucosa* with unilocular reproductive structures (left) and plurilocular reproductive structures (right) [after Tanaka and Chihara 1980b, p. 230, fig.2]; c. *R. hesperia* with unilocular reproductive structures (after Hollenberg 1969, p. 300, fig. 28); d. *R. pacifica* with plurangia and a hair pit [after Hollenberg 1969, p. 294, fig. 17]; e. *R. bornetii* with unilocular and plurilocular sporangia [after Kuckuck 1894, p.246, fig.15].

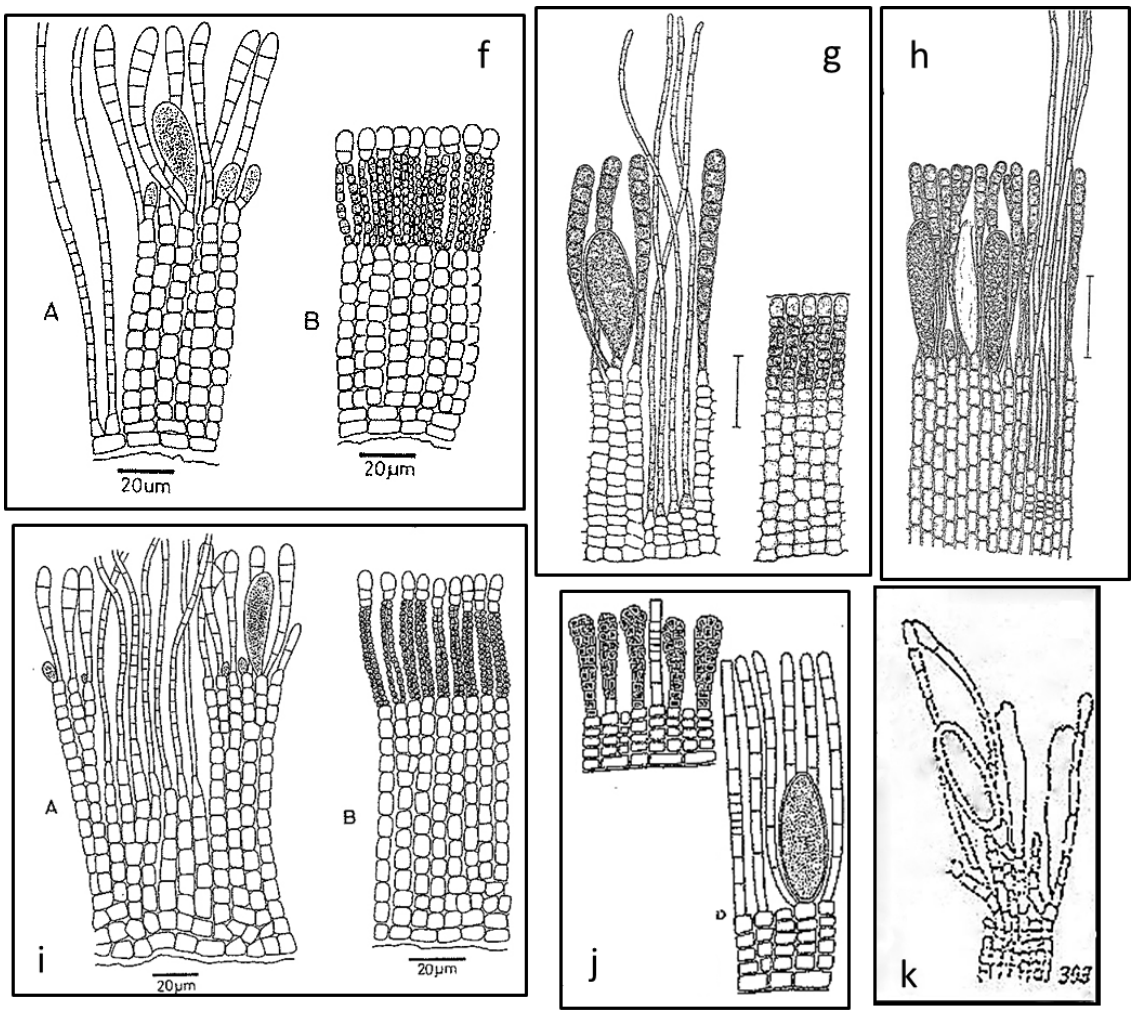

Figure 2.6 (continued) f. *R. pedicellata* showing unilocular (left) and plurilocular (right) reproductive structures (after Tanaka and Chihara 1981c, p. 103, fig. 4); g. *R. confusa* showing unilocular (left) and plurilocular (right) reproductive structures (after Abbott and Hollenberg 1976, p. 165, fig. 131); h. *R. integra* showing unilocular sporangia and a hair pit (after Hollenberg 1969, p. 294, fig. 13); i. *R. endopluroides* showing unilocular (left) and plurilocular (right) reproductive structures (after Tanaka and Chihara 1981c, p. 100, fig. 2); j. *R. tenuis* shown with plurilocular and unilocular reproductive structures (after Kylin 1947, p.44, fig. 38C - D); k. *R. longicellularis* showing unilocular reproductive structures (after Perestenko 1980, fig. 309).

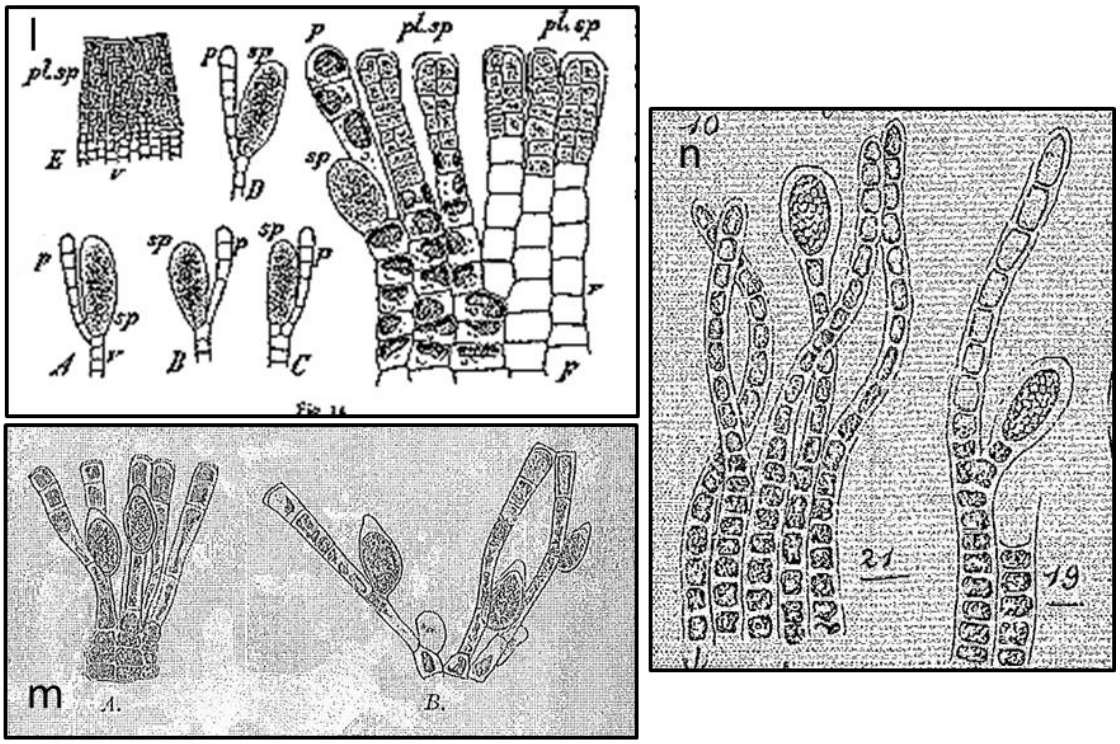

Figure 2.6 (continued) l. *R. clavata* with unilocular and plurilocular sporangia (after Kuckuck 1894, p. 244, fig. 14); m. *R. ovata* with unilocular sporangia (after Rosenvinge 1893, p. 900, fig. 30); n. *R. spongiocarpa* with terminal and lateral unilocular sporangia (after Batters 1890, pl. VIII, figs. 19 and 21).

2.4.2 Lithodermataceae Kjellman

Kjellman (1883) first established the family Lithodermataceae (as Lithodermateae) on the basis of the distinct lateral disposition of the plurilocular sporangia in *Lithoderma fatiscens* Areschoug whom the author illustrated in Plate 26, fig. 7. Several genera with terminally inserted reproductive structures have been placed in this family and they include *Lithoderma*, *Pseudolithoderma*, *Sorapion*, *Symphyocarpus*, *Petroderma* and *Heribaudiella*. These genera are distinguished based on a combination of characters which include the degree of association between erect filaments, number of plastids per cell, disposition of reproductive structures and organisation of sori. The systematic position of Lithodermataceae has been conflicting: Hollenberg (1969) included

Lithodermataceae in Ralfsiaceae, Taylor (1957) included it in the Chordariales, while Rosenvinge (1893, 1899) and Jonsson (1903) placed *Lithoderma* in the Myrionemaceae. Until now, no taxonomic conclusion can be made regarding the affiliation of this family until DNA sequence data is available for the type i.e. *Lithoderma fatiscens* Areschoug.

2.4.2.1 *Lithoderma* Areschoug

Generitype: *Lithoderma fatiscens* Areschoug (Fig. 2.7)

Type locality: west coast of Sweden - from the reefs of Gothenburg to Väderöarna and Koster.

Distribution: Sweden (Waern, 1949); East Greenland (Lund, 1959); Falkland Islands and the Antarctic (Skottsberg, 1921; 1953)

Areschoug (1875) established this genus after Kjellman presented him with plurilocular sporangia bearing plants of *L. fatiscens* although he had earlier referred to this species as *Ralfsia fatiscens* in his lecture. The diagnosis for this genus include: thalli thin, attached firmly to substratum; erect filaments simple, sparsely branched arising from the basal layer; reproductive structures arise from transformation of apical cells of the erect filaments; unilocular sporangia obovate, linear-oblong to elliptical-elongate; plurilocular sporangia issuing laterally from elongated-inflated filaments arising from the apical cells (Fig. 2.7) and several plastids per cell. In the same paper, Areschoug described *L. fatiscens* and the freshwater *L. fluviatile* without providing any illustrations (which later led to much debate) and he did not designate a generitype. Svedelius (1930) designated *L. fatiscens* Areschoug as the type species and transferred *L. fluviatile* to *Heribaudiella*. Illustrations for the generitype were first provided by Kjellman (1883); however the unilocular sporangia illustrated (plate 26, fig. 6) and ascribed to *L.*

fatiscens was considered by Waern (1949) and Skottsberg (1921) to belong to *Sorapion kjellmanii* (Wille) Rosenvinge and *Petroderma maculiforme*, respectively. This is not impossible since both types of reproductive structures occur on different thalli. Later, Hauck (1885) described and illustrated *L. fatiscens* from Helgoland with lateral plurilocular sporangia and terminal unilocular sporangia. Waern (1949) highlighted that the unilocular sporangia illustrated by Kjellman (1883) and Hauck (1885) and ascribed to *L. fatiscens* were markedly different.

There has been much confusion between species of *Lithoderma* and *Pseudolithoderma* which were distinguished by the position of their plurilocular reproductive structures (lateral in *Lithoderma* and terminal in *Pseudolithoderma*). The '*Lithoderma* problem' (Waern, 1949) first started when Kuckuck (1894) described a different form of plurilocular sporangia (see Fig. 2.8a) from Helgoland which he ascribed to *L. fatiscens*. Although he mentioned the possibility of his observed form to be identical with *Ralfsia extensa* Crouan & Crouan (which he dismissed on the grounds of difference in habit), Kuckuck went further (with Bornet sharing his view) by amending the diagnosis of *Lithoderma* and the type species, *L. fatiscens* to exclude Areschoug's lateral plurilocular sporangia and substituted it with his terminal type; this was despite Kjellman's suggestion to Kuckuck (via their mail correspondence) to set up another genus for his observed form. However, it should be noted that Kuckuck raised a possibility that Areschoug's lateral plurilocular sporangia may be a third kind of sporangia in *L. fatiscens*, in other words dimorphism of plurilocular sporangia such as in *Giraudia*. The connecting link lies in the terminal unilocular sporangia described by both Areschoug (not illustrated) and Kuckuck (see Fig. 2.8a). According to Waern (1949) who examined Areschoug's original material, Areschoug's plant with terminal unilocular sporangia does not represent the one illustrated by Kuckuck (1894). Lund (1938) attempted but was unsuccessful in solving the relationship between *L. fatiscens*

Areschoug and *L. fatiscens* Kuckuck based on East Greenlandic and Danish materials. This matter was considered resolved when Svedelius (in Kjellman and Svedelius, 1910) founded a new genus *Pseudolithoderma* to accommodate species with both types of reproductive structures in terminal position. Most of the species initially included in *Lithoderma* has been transferred to other genera such as *Pseudolithoderma* (see below), *Petroderma* (*L. maculiforme*), *Sorapion* (*L. simulan*s, *L. kjellmanii*) and *Heribaudiella* (*L. zonata, L. fluviatile, L. fontanum*). Hamel (1935) and later, Hollenberg (1969) who examined the original specimen of *Ralfsia extensa* collected from Brest (France) and described by the Crouan brothers (1867), found that it was identical with the description of *L. fatiscens* with terminal plurilocular sporangia by Kuckuck (1894). *Lithoderma extensum* (Crouan frat.) Hamel was subsequently transferred to *Pseudolithoderma* by Lund (1959).

Until now, the real unilocular sporangia of *Lithoderma fatiscens* Areschoug has yet to be determined. Waern (1949) who examined the original slides by Areschoug found that mixtures of four species were identified from Areschoug's collection. They include *L. fatiscens* Areschoug for the lateral plurilocular sporangia, *L. extensum* (Crouan frat.) Hamel for the terminal plurilocular sporangia, *Sorapion kjellmanii* for the unilocular sporangia observed by Areschoug and a new species which he described in the same paper i.e. *Lithoderma rosenvingei* Waern for the terminal four-parted unilocular sporangia. Apparently, the terminal type of plurilocular sporangia was also detected by Kylin (1907) in Kjellman's set of slides. Waern (1949) reported that the terminal unilocular sporangia described by Areschoug are surrounded by free projecting filaments with elongated cells and one plastid in each cell; thus it was interpreted as belonging to *S. kjellmanii*. Batters (1896) was probably right when the author suggested the similarity between *Lithoderma* and *Sorapion* in terms of their unilocular sporangia, and proceeded to transfer *S. simulans* to *Lithoderma*. Rosenvinge (1899) commented

that the plurilocular sporangia of *L. fatiscens* Areschoug were inserted in a similar manner as the unilocular sporangia of *Ralfsia ovata* and thus suggested the '*Ralfsia ovata* hypothesis' in which the two might belong to the same species. However, Rosenvinge's *R. ovata* was reported with a single chloroplast per cell while Lund (1938) noticed that *L. fatiscens* with lateral plurilocular sporangia possess several plastids in each cell.

Kjellman (1883) described a new species, *Lithoderma lignicola* based on sterile materials from the Norwegian Sea. Several authors including Waern (1949, 1952), Wilce et al. (1970) and Fletcher (1987) regarded this species as a sterile old crust of *P. maculiforme* based on its loosely united erect filaments, construction of thallus, cell proportions and measurements and possession of a single chloroplast per cell. Hauck (1885) described *Lithoderma adriaticum* from the Adriatic Sea which he distinguished from *L. fatiscens* Areschoug on the basis of its thicker and darker colour thallus. It is unsure whether this is indeed a distinct species; Fletcher (1987) suggested it to be conspecific with *Pseudolithoderma roscoffense* but Hauck's description included unilocular sporangia similar to *L. fatiscens* while plurilocular sporangia were unknown (both features were contradictory to the description of *P. roscoffense*). Verlaque (1988) transferred *L. adriaticum* to *Pseudolithoderma*. Waern (1949) described *Lithoderma rosenvingei* (Fig. 2.8h) and *Lithoderma subextensum* (Fig. 2.8g) which are frequently intermixed based on materials from the east coast of Sweden. The former was distinguished by its unique terminal unilocular sporangia in fours; easily separated erect filaments; absence of hairs and small numerous (seven to eight) chloroplasts per cell whereas the latter species was described with hair; erect filaments not easily separated by pressure; unilocular sporangia terminal, elongated cylindrical often with waist; plurilocular sporangia terminal, uniseriate and branched (different from *L. extensum*); both types of reproductive structures occurring on the same plant and a few (three to

four) comparatively large chloroplasts per cell. The peculiar sporangia in *L. rosenvingei* was earlier described from East Greenland by Rosenvinge (1899) as young unilocular sporangia of *L. fatiscens* and a small part of this species was seen in Areschoug's slide. Lund (1959) later transferred both species to *Pseudolithoderma*. Skottsberg (1921) described *Lithoderma piliferum* from the Falkland Islands on the basis of presence of hairs, numerous disc-shaped plastids, closely adherent erect filaments and terminal subglobose unilocular sporangia while plurilocular sporangia were unknown; later in 1953 the same author described *Lithoderma antarcticum* from the Antarctic. Algaebase (Guiry and Guiry, 2013) listed this genus under the family Ralfsiaceae, however the systematic position of this genus and the family Lithodermataceae is generally treated as *incertae sedis* pending the sequence data of the type species.

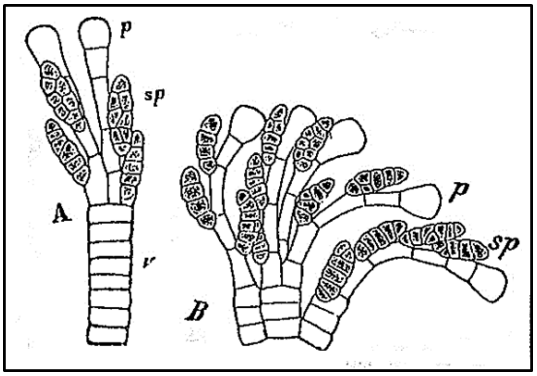

Figure 2.7 *Lithoderma fatiscens* Areschoug shown with lateral plurilocular reproductive structures (after Kuckuck 1894, p. 238, fig. 12)

2.4.2.2 *Pseudolithoderma* Svedelius (Fig. 2.8)

Holotype: *Pseudolithoderma fatiscens* (Kuckuck) Svedelius

Taxonomic synonym and currently accepted name for type species: *Pseudolithoderma extensum* (Crouan frat.) Lund

Type locality: Helgoland, Germany

Distribution: Largely confined to the North Atlantic Ocean (e.g.: Waern, 1949; Taylor, 1957; Sears and Wilce, 1973; Lee, 1980; Fletcher, 1987; Ribera et al., 1992; Parente et al., 2000). Others include Japan (Tanaka and Chihara, 1981c); southern Australia (Womersley, 1987); India (P. Sophiamal Nettar and M.V.N. Panikkar, 2009), Hong Kong (Kaehler, 1998), Pacific coast of North America (Hollenberg, 1969), Argentina (Asensi and Küpper, 2012), New Zealand (Harper et al., 2012)

This genus is erected by Svedelius (in Kjellman and Svedelius, 1910) based on *L. fatiscens sensu* Kuckuck which possess terminal plurilocular sporangia, uncapped by sterile apical cells. Other generic characteristics include relatively thin thalli, firmly adherent to substratum without rhizoids; erect filaments simple or sparsely branched arising from a thin primarily one to two-celled thick basal layer, cells containing several chloroplasts without pyrenoids and unilocular sporangia terminally borne on the erect filaments. Species of this genus are distinguished by a combination of the following features: organisation and shape of reproductive structures, the degree to which erect filaments adjoin to each other and texture of the crust (soft or rigid). The type species is mainly distinguished by its uniseriate (to eight loculi, each locule empties on its own) attenuates at the apex plurilocular sporangia with oblique cross walls (see Fig. 2.8a), slightly globose unilocular sporangia, firmly adjoined filaments and firm crust. Culture studies by Kuckuck (1912) indicate that this species undergoes an isomorphic life history in which the plurispores behave like isomorphic gametes with sexual fusion occurring to produce a zygote. Nevertheless, mature crust with unilocular sporangia was not produced from these zygotes and cytological data was lacking.

Pseudolithoderma roscoffense (Fig. 2.8f, as *P. roscoffensis*) Loiseaux (1968) was first described from Roscoff (France) and distinguished from other species by its firm and smooth crust, closely adjoined erect filaments, biseriate to multiseriate

plurilocular sporangia with straight cross walls while unilocular sporangia were unknown. Loiseaux (1968) and Fletcher (1978) who conducted culture studies on *P. roscoffense* suggest its life history to be of the 'direct' type whereby swarmers from plurilocular bodies germinate directly without sexual fusion and gave rise to plurilocular structures-bearing crustose thallus. Fletcher (1987) reported that the immature plurilocular sporangia of this species resemble paraphyses or unilocular sporangia. *Pseudolithoderma nigrum* Hollenberg (Fig. 2.8e, as *P. nigra*) represents the first species of *Pseudolithoderma* reported from the Pacific. It was reported with filamentous early development and suspected to lack alternation of generations. This species is characterised by its firmly adjoined erect filaments and terminal plurilocular sporangia with paraphysis-like cells while unilocular sporangia were unknown. Hollenberg (1969) distinguished his alga from *P. roscoffense* which shared similar plurilocular sporangia by the size and colour of thalli crust (less than 3 cm broad yellowish brown crust in *P. roscoffense*; 2 - 10 cm or more broad dark brown to black crusts in *P. nigrum*) and the absence of paraphysis-like structures in *P. roscoffense*, features which in my opinion, are insufficient for species delineation as they tend to vary between specimens from different localities. In addition, the paraphysis-like structures of *P. nigrum* may be what Fletcher (1987) termed as immature plurilocular sporangia. Another species suspected to be conspecific with *P. roscoffense* is *P. thangasseriense* (Fig. 2.8b, as *P. thangasseriensis*) Sophiamal Nettar & Panikkar described from the coast of Kerala which marks the first report of this genus from Asia. This species was described with terminal plurilocular sporangia not unlike those of *P. roscoffense* (although there was no mention of paraphysis-like structures), compactly arranged erect filaments and lack of unilocular sporangia.

Sears and Wilce (1973) described *P. paradoxum* (Fig. 2.8d) from the Atlantic coast of North America and included it in the Ralfsiaceae under Ectocarpales. This

species is given the paradoxum epithet due to its characteristics which are intermediate between *Petroderma* and *Pseudolithoderma*. Its soft, almost spongy crust; easily separated erect filaments and cylindrical terminal unilocular sporangia with collar-like remnants are features of *Petroderma*, and yet each of its cells contains several plastids which led to its inclusion in *Pseudolithoderma*. Sears and Wilce (1973) probably did not include their species under *Lithoderma* due to the absence of soral paraphyses in their alga, which they use as a distinguishing feature of the two genera (the soral paraphyses described by Areschoug for *L. fatiscens* has been identified as belonging to *S. kjellmanii*; Waern (1949) has also described *L. fatiscens* to have rather easily separated erect filaments). *Pseudolithoderma paradoxum* differs from other species of its genus by its soft texture; absence of gelatinous layer in the crust surface (as is the case with *P. nigrum* and *P. australe*); shape and organisation of unilocular sporangia; and unknown plurilocular sporangia: *P. paradoxum* shares a similarity of easily separated filaments with *P. rosenvingei* but both can be distinguished based on their unilocular sporangia and the presence of gelatinous covering in the latter species; *P. paradoxum* can be easily differentiated from *P. extensum* based on the latter's closely adjoined erect filaments, presence of gelatinous membrane and globose unilocular sporangia. The difference between *P. paradoxum* and *P. subextensum* lies in the former's loosely adjoined erect filaments, larger number of plastids per cell and absence of plurilocular sporangia. Meanwhile it is distinguished from *P. roscoffense* on the basis of the latter's rigid thallus, absence of unilocular sporangia and possession of a gelatinous covering.

Womersley (1987) described *P. australe* (Fig. 2.8c, as *P. australis*) from southern Australia and characterised it by erect filaments separating moderately readily under pressure; large cylindrical to clavate unilocular sporangia sunken within the thallus at various levels (in other species, the unilocular sporangia form a surface layer)

and uniseriate plurilocular sporangia, oblong-ovoid with five to eight loculi. *Pseudolithoderma moreirae* Yoneshigue & Boudouresque reported from Brazil as part of Yoneshigue's doctoral thesis (Yoneshigue, 1985) was considered invalid due to the lack of Latin diagnosis. Tanaka and Chihara (1981c) reported the presence of *P. subextensum* in Japan, marking the first record of this genus in the country. However, their description somewhat differed from the original description by Waern (1949) whereby hairs arise from the basal layer in their alga (in *P. subextensum*, hairs arise from the terminal cell of erect filaments), the two types of reproductive structures occur on separate thalli (in *P. subextensum*, both types of reproductive structures are sometimes present on the same thallus) and the presence of ascocyst-like paraphyses associated with unilocular sporangia (not mentioned in *P. subextensum*). On top of that, Tanaka and Chihara's illustration of the plurilocular sporangia of their alga bears a closer resemblance to *P. roscoffense* rather than the ramified ones in *P. subextensum*. Despite Kaehler's (1998) report of *P. subextensum* from Hong Kong, the author only provided a micrograph of the unilocular sporangia but not the characteristically ramified plurilocular sporangia, thus leading to a speculation of the identity of his alga.

In a molecular study by Bittner et al. (2008), *P. roscoffense* was resolved as a sister of the order Sphacelariales. However, Parente et al. (2005; unpublished results presented at the VIIIth International Phycological Conference in Durban) indicated that the type of the genus i.e. *P extensum* is not closely related to *P. roscoffense*, further complicating the systematic position of this genus as a whole.

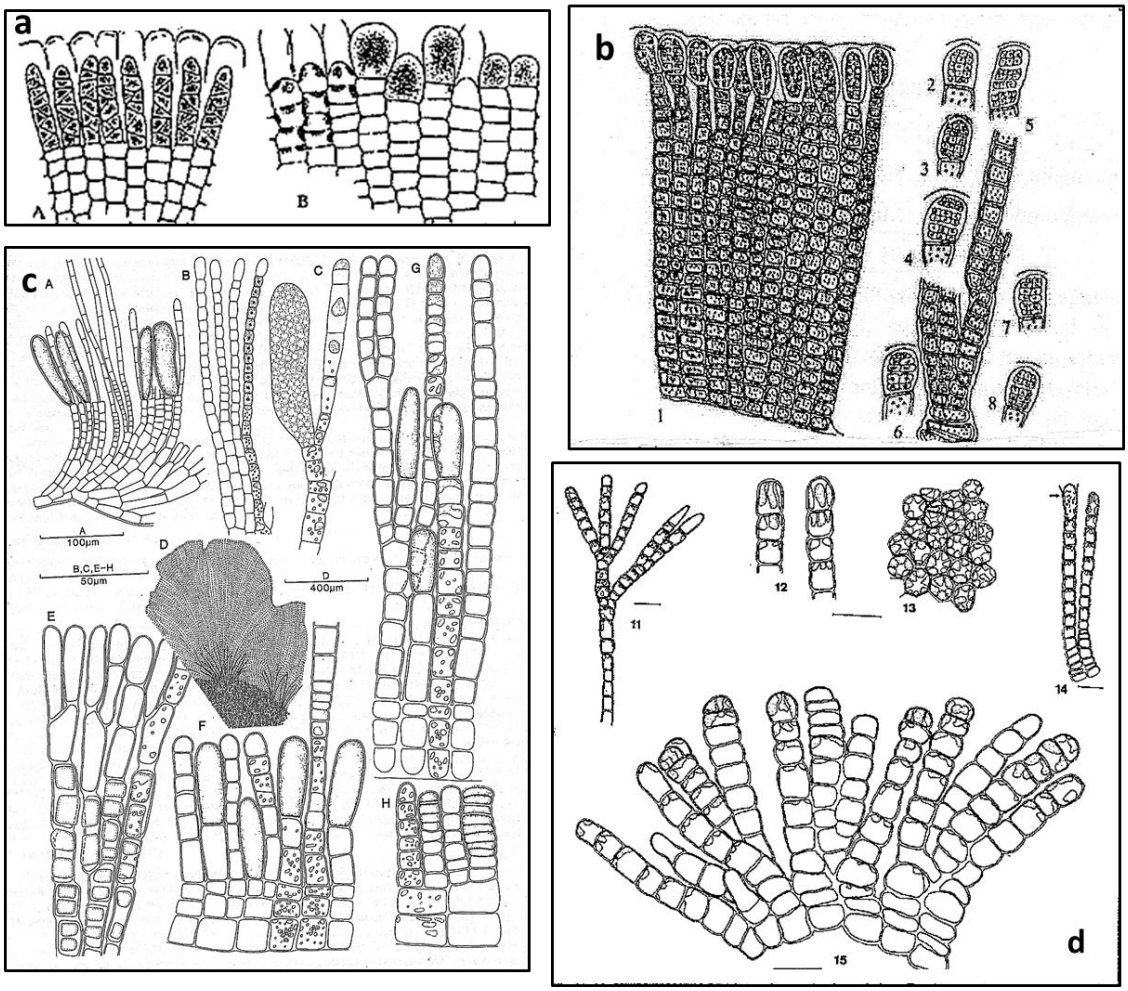

Figure 2.8 *Pseudolithoderma* spp. a. *P. extensum* with terminal plurilocular sporangia (left) and unilocular sporangia (right) [after Kuckuck 1894, p. 238, fig. 1]; b. *P. thangasseriense* with terminal plurangia at different stages of development (after Sophiammal Nettar and Panikkar 2009, p. 9, figs. 1 - 8); c. *P. australe* with unilocular sporangia (labelled F and G) and plurilocular sporangia (labelled H) [after Womersley 1987, p. 71, fig. 19]; d. *P. paradoxum* shown with unilocular sporangia (after Sears and Wilce 1973, p. 80, figs. 11 - 15).

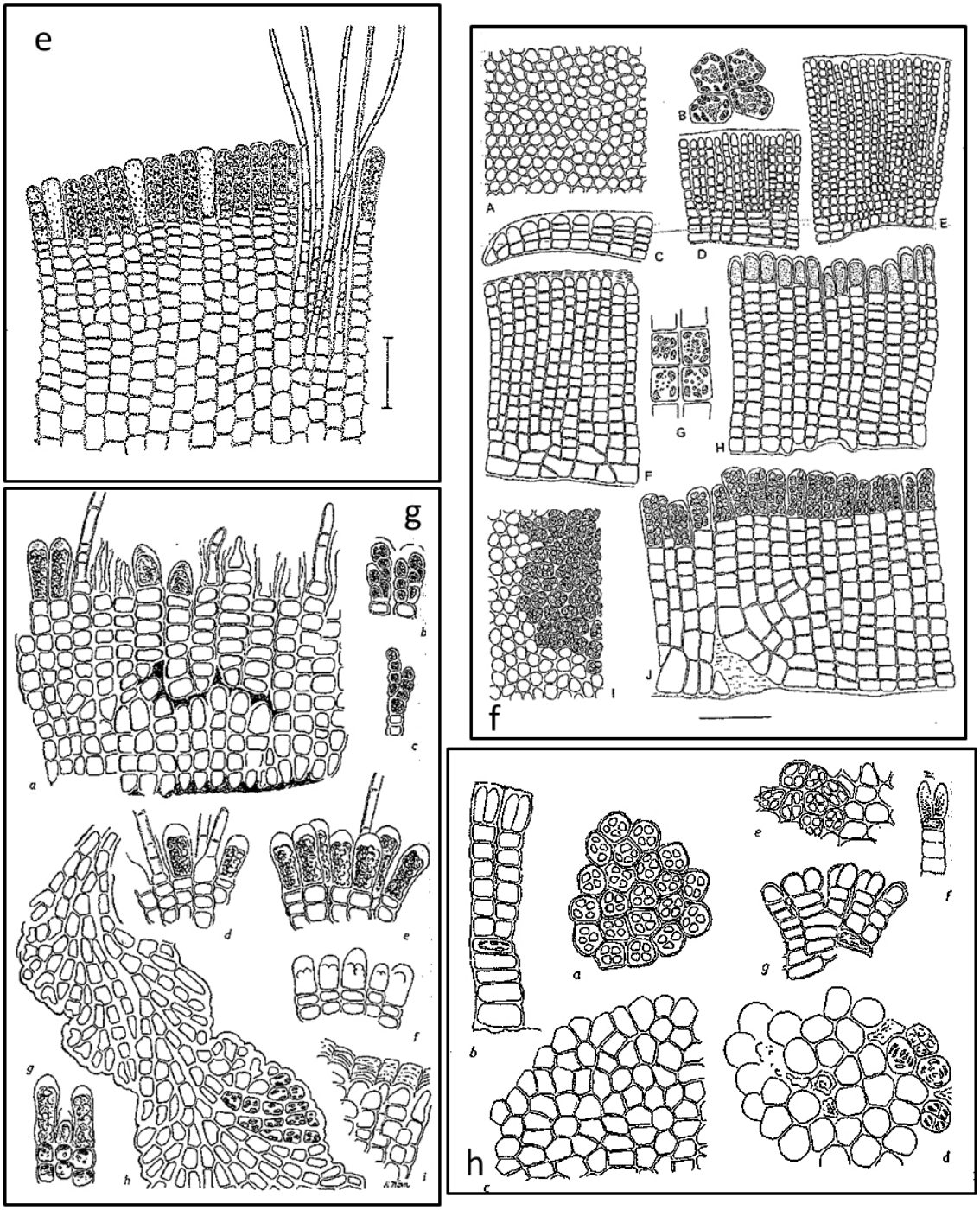

Figure 2.8 (continued) e. *P. nigrum* showing plurangia and hair pit (after Hollenberg 1969, p. 299, fig. 19); f. *P. roscoffense* with cells having several discoid chloroplasts (labelled G) and terminal plurilocular sporangia (labelled J) [after Fletcher 1987, p. 87, fig. 3]; g. *P. subextensum* with terminal unilocular sporangia and the ramified terminal plurilocular sporangia (after Waern 1949, pg. 660, fig. 4); h. *P. rosenvingei* with four-parted unilocular sporangia seen from the top and from the vertical section (after Waern 1949, pg. 656, fig. 3).

2.4.3 Nemodermataceae Kuckuck ex Feldmann

This family was proposed by Kuckuck (1912) on the basis of its unique reproductive structures i.e. laterally inserted plurilocular reproductive structures and intercalary unilocular reproductive structures. This family is distinguished from the Ralfsiaceae in having numerous discoid chloroplasts without pyrenoids and an isomorphic life history with marked anisogamy (Kuckuck, 1912), rather than a single plate-like plastid lacking pyrenoids and a direct life cycle. Currently, this family is represented by the monotypic genus *Nemoderma* with the type species, *N. tingitanum*.

2.4.3.1 *Nemoderma* Schousboe ex Bornet

Generitype: *Nemoderma tingitanum* Schousboe ex Bornet 1892 (Fig. 2.9)

Type locality: Morocco, Mediterranean

Distribution: Common in (sub) tropical regions of the Atlantic Ocean and Mediterranean Sea - France (Feldmann, 1937); Italy, Spain, Algeria, Tunisia (Ribera et al., 1992); Canary Islands (John et al., 2004); Salvage Islands (Parente et al., 2006); Azores (Parente et al., 2000).

The type species is easily distinguished by its soft, gelatinous and yellowish-brown crust of which erect filaments, easily separable under light pressure arise from a discoid base; cells with several discoid or irregular chloroplasts; intercalary unilocular sporangia and laterally inserted gametangia (oogonia and antheridia). According to Hamel (1935), the antheridia and oogonia occur on the same thallus with the antheridia generally located below the sessile or stalked oogonia while unilocular sporangia occur on different individuals. A life history involving sexuality has been established for this

genus in which heterogamous copulation of female gametes and antherozoids and germination of zygotes; zoospores and also female gametes that can germinate without copulation, growing directly back to a disc similar to the adult individuals, were observed (Kuckuck, 1912). Fletcher (1978) was right when he speculated that *Nemoderma* was "a possible example of an advanced plant in an evolutionary line of soft crusts starting from a *Petroderma*-like ancestor". A molecular study by Phillips et al. (2008) showed a distant relationship between *N. tingitanum* and the rest of the crustose brown algae and this monotypic taxon is currently accorded its own order, the Nemodermatales.

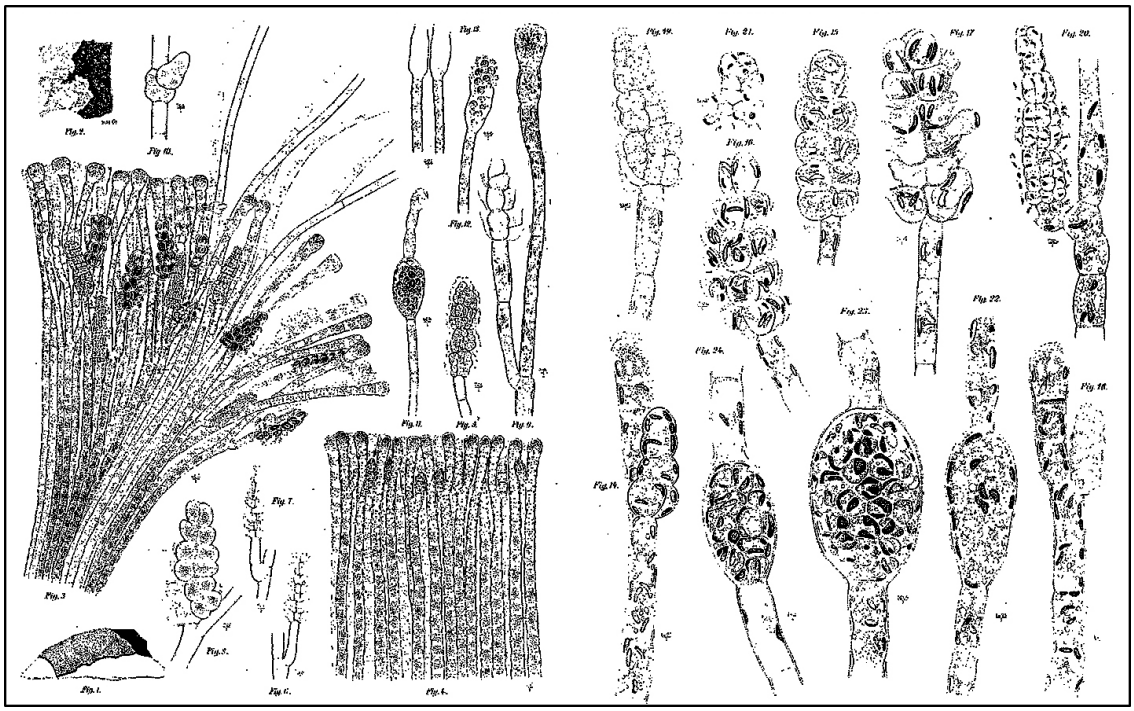

Figure 2.9 *Nemoderma tingitanum* with antheridia, oogonia and unilocular sporangia (after Kuckuck 1912, pp. 149 and 151, pl. IV and V)

2.4.4 Mesosporaceae Tanaka & Chihara

Tanaka and Chihara (1982) erected the family Mesosporaceae which comprise *Mesospora* as the type genus and two other closely related genera, *Hapalospongidion* and *Basispora*. The same authors placed Mesosporaceae in Ralfsiales and distinguished it from other families of the order by the intercalary plurilocular reproductive structures and terminal unilocular reproductive structures arising on a stalk or on a vegetative filament without associated paraphyses. The three genera included in this family also shared a similarity in having free, erect filaments laterally cohesive in a gelatinous matrix.

2.4.4.1 *Hapalospongidion* Saunders (Fig. 2.10)

Generitype: *Hapalospongidion gelatinosum* Saunders

Type locality: Pacific Grove, California, North America

Distribution (excluding species transferred from *Mesospora* and *Basispora*): North America (Hollenberg, 1942), New Zealand (Buchanan, 2005), Western Australia (Womersley, 1987), India (Sophiammal Nettar and Panikkar, 2009), Hong Kong (Kaehler, 1998)

The genus *Hapalospongidion* was established by Saunders (1889) for *H. gelatinosum* from California. Lindauer (1949) subsequently added two new species from New Zealand to the genus, *H. saxigenum* Lindauer and *H. durvillaeae* Lindauer, but they were later transferred to the genus *Basispora* by John and Lawson (1974). Womersley (1987) later made a new addition to this genus with *H. capitatum* Womersley from Australia. Another species, *H. thirumullavaramense* P. Sophiammal Nettar & M. V. N.

Panikkar was reported from Kerala, India in 2009. The *H. pangoense* described by Abbott and Huisman (2004) is probably a species of *Mesospora* or even *Ralfsia*, but it is unlikely a species of *Hapalospongidion* on the basis of its thallus construction and number of stalk cells (up to four) from which unilocular sporangia arise.

Hapalospongidion is characterised by its cushion-like gelatinous thallus (*apalos* meaning soft; *spongos* meaning "a sponge" in Greek) composed of long erect and mostly unbranched filaments with a large number of cells (more than 40; up to 60 cells in both *H. saxigenum* and *H. capitatum*; up to 85 cells in *H. thirumullavaramense*) arising from a basal plate; plurilocular sporangia borne near the apex of erect filaments and unilocular sporangia arising from the transformation of terminal cell of the shorter erect filament. Unilocular sporangia were not observed for *H. capitatum* and *H. thirumullavaramense*. Lindauer (1949) distinguished *H. saxigenum* from the type species on the basis of the former's absence of plurilocular bodies and in the poorly developed basal layer. Nonetheless, Buchanan (2005) reported plurilocular sporangia for his New Zealand specimens and concluded that *H. saxigenum* is identical to *H. gelatinosum* because the New Zealand material is consistent with the description of *H. gelatinosum*. Womersley (1987) distinguished *H. capitatum* from the type species based on the larger, capitate, uppermost two to three cells of the erect filaments and in having more elaborate plurilocular sporangia; and from *H. saxigenum* which does not have the capitate erect filaments and is only known with unilocular sporangia. However, Buchanan (2005) commented that the multiseriate plurilocular reproductive structure was the only consistent character to distinguish between *H. capitatum* and *Hapalospongidion* from New Zealand. He mentioned that the feature of capitate filaments in *H. capitatum* is variable and was seen in some of the New Zealand specimens. The *H. macrocarpum* (Feldmann) León Álvarez & González-González

reported by Parente et al. (2006) from the Salvage Islands, Madeira Archipelago is likely to be *H. saxigenum* based on the given description and illustration (p. 22, fig. 3).

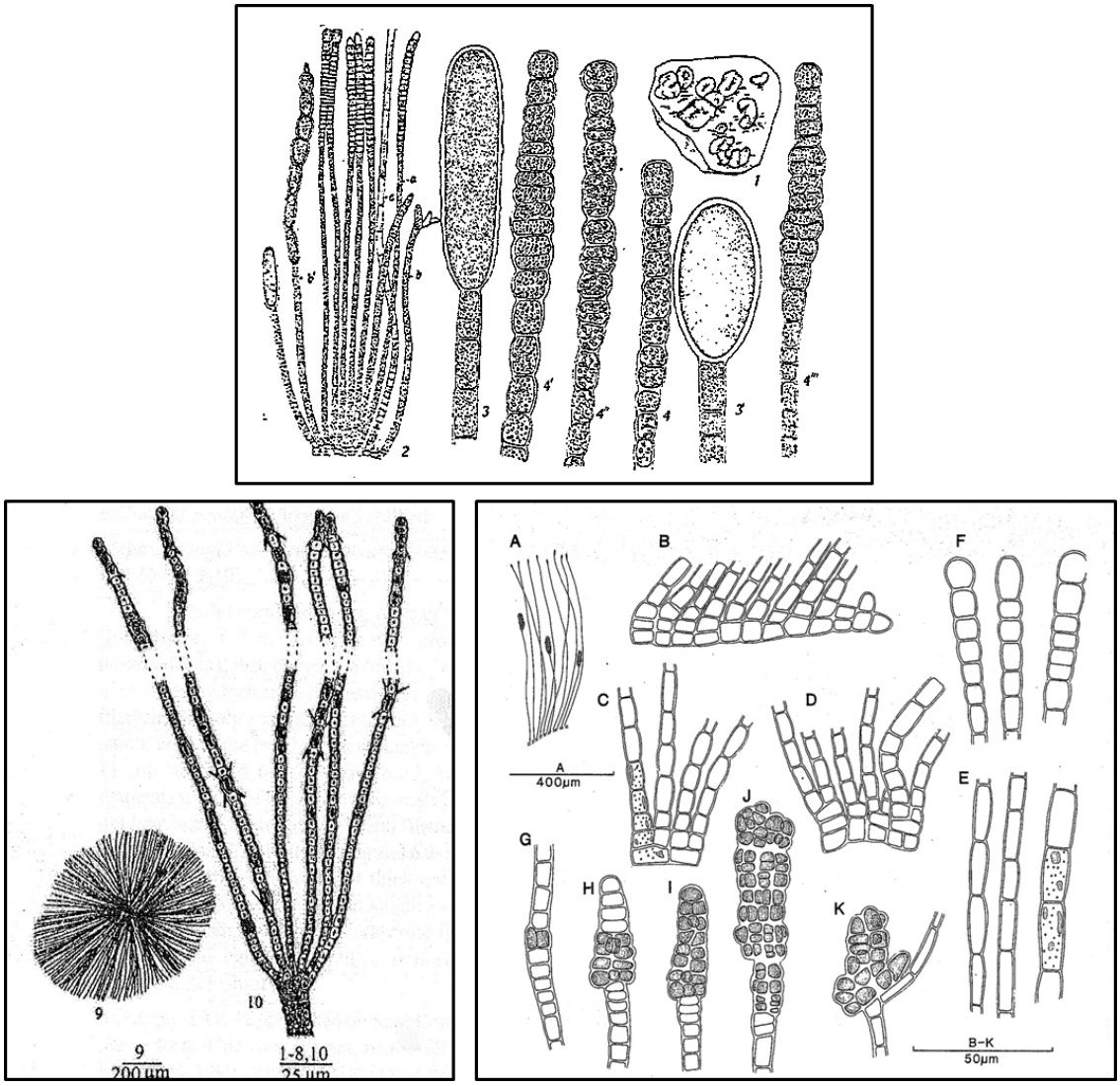

Figure 2.10 *Hapalospongidion* spp. Top: *H. gelatinosum* with unilocular and plurilocular sporangia (after Saunders 1899, plate 1: figs. 1 - 4), bottom left: *H. capitatum* with plurilocular sporangia (after Womersley 1987, p. 76, fig. 20), bottom right: *H. thirumullavaramense* with plurilocular sporangia (after Sophiammal Nettar and Panikkar 2009, p. 9, figs. 9 - 10).

2.4.4.2 *Mesospora* Weber-van Bosse (Fig. 2. 11)

Generitype: *Mesospora schmidtii* Weber-van Bosse

Type locality: Weber-van Bosse (1911) did not designate a holotype; the syntype localities were all situated in Indonesia

Distribution: Aside from *M. macrocarpa* which was reported from the Mediterranean, other species of this genus were reported in warm waters of the Indian Ocean and the (sub) tropical western Pacific Ocean

This genus is characterised by having a mucilaginous thallus adherent to the substratum without rhizoids, loosely adherent and clavate erect filaments consisting up to 30 cells, intercalary plurilocular sporangia borne near the apex of erect filaments and unilocular sporangia terminally borne on stalk cells and lateral to the erect filaments, unaccompanied by paraphyses. Weber-van Bosse (1911, 1913) initially placed *Mesospora* in the Ralfsiaceae with *M. schmidtii* as the type species albeit with some uncertainty in view of the thin horizontal basal layer which consisted of generally two and rarely four layers of cells. In the protologue, Weber-van Bosse (1911) did not designate a holotype among specimens collected from the original six syntype localities. A microslide preparation which was part of the original material of *M. schmidtii* was recently designated as the lectotype (Poong et al., 2013).

Currently, six species are recognised and these include: *M. schmidtii*, *M. vanbosseae* Børgesen (1924) from Easter Island, *M. macrocarpa* (Feldmann) den Hartog (1968) from the Mediterranean, *M. pangoensis* (Setchell) Chihara & Tanaka from Tutuila Island and its variety, *M. pangoensis* var. *galapagensis* (Setchell & Gardner) Chihara & Tanaka (Tanaka and Chihara, 1982) from the Galapagos Islands, *M. negrosensis* West & Calumpong (1996) from the Philippines and the recently

described *M. elongata* Poong, Lim & Phang (Poong et al., 2013) from Japan and Lombok Island which was partially derived from results of the current study (see Discussion for species delineation in *Mesospora*).

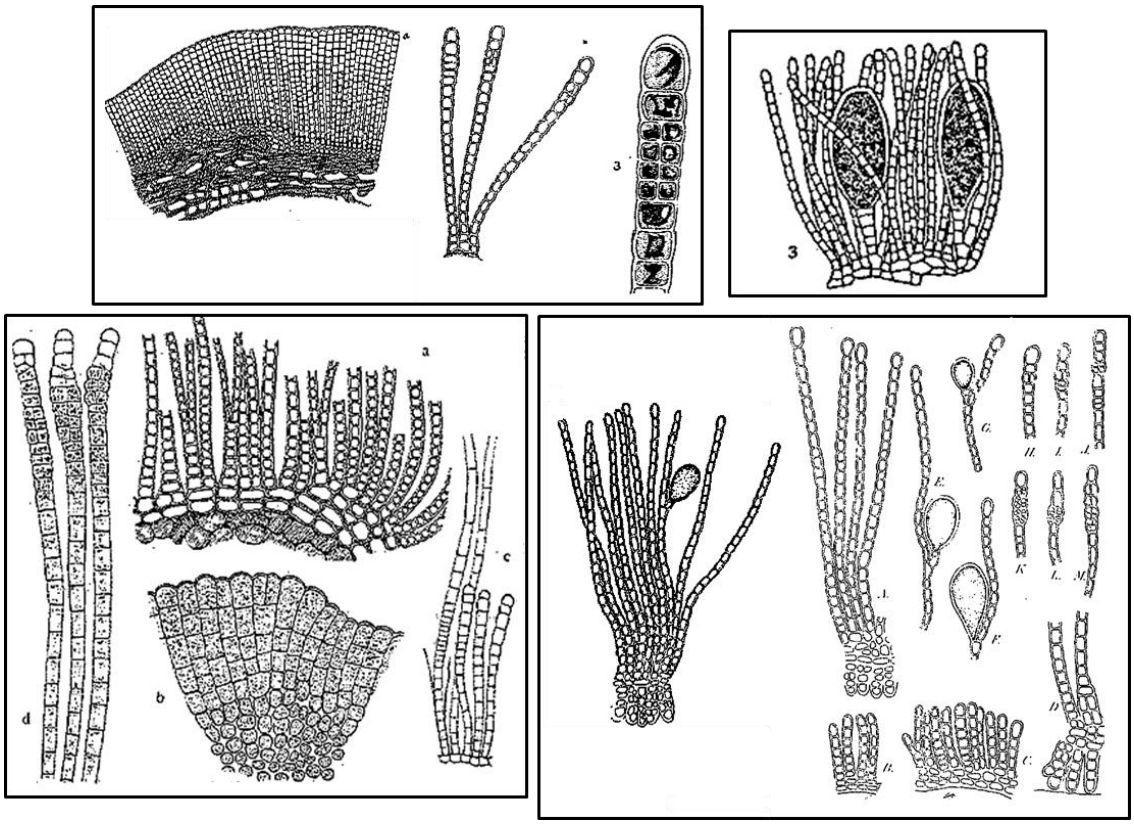

Figure 2.11 *Mesospora* spp. Top left: *M. schmidtii* with plurilocular sporangia (after Weber-van Bosse 1913, p. 143, fig. 43 and plate II: figs. 2 - 3), top right: *M. pangoensis* with unilocular sporangia (as *Ralfsia pangoensis*, after Setchell 1924, p.167, fig. 33- no. 3), bottom left: *M. vanbosseae* with plurilocular sporangia (after Børgesen 1924, p. 259, fig.9), bottom right: *M. macrocarpa* with unilocular and plurilocular sporangia (as *M. mediterranea*, after Feldmann 1937, pp. 264-265, figs. 40 - 41).

2.4.4.3 *Basispora* John & Lawson

Generitype: *Basispora africana* John & Lawson (Fig. 2.12)

Type locality: Just to the west of the village of Kokrobite (5° 29’ N., 0° 21’ W.), Ghana. Growing in the lower part of the upper subzone of the eulittoral, on rocks moderately exposed to wave-action where the barnacles are in low abundance.

Distribution: West African coast - Angola, Cameroon, Côte d'Ivoire, Gambia, Ghana, Liberia, Namibia, Senegal, Sierra Leone (John et al., 2004)

This genus was established and distinguished from other members of the family Ralfsiaceae by the terminal unilocular reproductive structures arising on long stalks of (4-) 6 - 10 (-15) cells from near the base of the simple, often assurgent laterally free vegetative filaments. *Basispora* was thus named to signify the position on the erect filaments from which the sporangia arise. John and Lawson (1974) transferred two species of *Hapalospongidion* i.e. *H. saxigenum* and *H. durveillaeae* to *Basispora* which lead to the combinations *B. saxigena* (Lindauer) John & Lawson and *B. durvillaeae* (Lindauer) John & Lawson, respectively. The latter was pointed out to be different from the other two species due to its parasitic nature. In the same year, South (1974) erected the genus *Herpodiscus* with *Herpodiscus durvillaeae* (Lindauer) South as the type and the alga was referred tentatively to Elachistaceae of the Chordariales. A molecular study by Heesch et al. (2008) has shown that this parasitic taxon (the host being *Durvillaea antarctica*) is affiliated with the Sphacelariales. John et al. (2004) cited *Hapalospongidion spongiosum* (sic) Saunders as a synonym of *B. africana*.

Basispora africana is reported with thallus consisting of three to several layers of prostrate filaments bearing assurgent filaments, free and closely packed, consisting up to 40 cells, distinctly clavate towards the upper end and several discoid chloroplasts

per cell while plurilocular reproductive structures were unknown. *Basispora*, like its two closely related genera, *Hapalospongidion* and *Mesospora*, all have erect filaments loosely attached to one another by mucilage and readily separate on squashing.

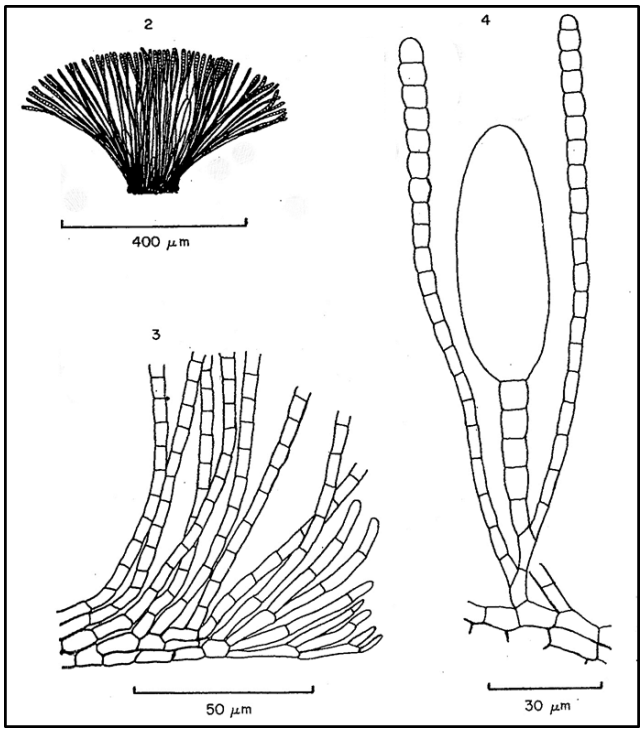

Figure 2.12 *Basispora africana* with unilocular sporangia (after John and Lawson 1974, p. 288, figs. 2 - 4).

2.4.4.4 Synonymisation of *Hapalospongidion*, *Mesospora* and *Basispora*

The genus *Mesospora* has been listed as a synonym of *Hapalospongidion.* Womersley (1987) regarded *Mesospora* and *Basispora* to be closely related to *Hapalospongidion* on the basis of chloroplast number and position of unilocular sporangia and thus, should be relegated to synonymy. In accordance to Womersley (1987), Silva et al. (1996) transferred *M. schmidtii* to *Hapalospongidion* and later León-Alvarez and González-González (1993) did the same for *M. macrocarpa* and *M. vanbosseae*. Although the

name *H. pangoense* was proposed by Silva et al. (1996) for *M. pangoensis* (Setchell) Chihara & Tanaka, this combination had previously been made by Hollenberg (1942) who misspelled the epithet as *pangoensis*. According to Womersley (1987), both *Mesospora* and *Hapalospongidion* shared the similarities of having one to three chloroplasts per cell whose number is probably dependent on the size and age of the cells and unilocular sporangia terminal on vegetative filaments which may or may not have branched close to the base. Meanwhile, *Basispora* was deemed similar to *Hapalospongidion* in possessing several chloroplasts per cell and terminal unilocular sporangia arising on long stalks from near the base of the laterally free erect filaments. In fact, a number of authors (John and Lawson, 1974; Tanaka and Chihara, 1982) have pointed out the close relationship between the aforementioned three genera which share the following combination of characters: crustose plants with a base of prostrate filaments from which simple, loosely adjoined filaments arise; intercalary plurilocular sporangia and unilocular sporangia terminal (origin) on erect filaments. Womersley (1987) retained the combined genus in Ralfsiaceae but a molecular study on members of the Ralfsiaceae and Mesosporaceae (Lim et al., 2007) has proven the independence of the two families and thus should the three genera be combined, the combined genus should be placed in the Mesosporaceae instead of the Ralfsiaceae.

2.4.5 Neoralfsiaceae Lim & Kawai

Lim et al. (2007) established this monotypic family to accommodate the new genus *Neoralfsia*, on the basis of *Ralfsia expansa*, which forms a distinct clade separated from members of the Ralfsiaceae.

2.4.5.1 *Neoralfsia* Lim & Kawai

Generitype: *Neoralfsia expansa* (J. Agardh) Lim & Kawai ex Cormaci & Furnari (Fig. 2.13a)

Type locality: Veracruz, Mexico (as *Myrionema expansum* J. Agardh)

Distribution: Widespread in warm temperate and tropical seas of both hemispheres. North America: León-Alvarez and González-González (2003), South America: Ramírez and Santelices (1991), Europe: Ribera et al. (1992), Africa: Rull Lluch (2002); John et al. (2004), Asia: Lim et al. (2007), Poong et al. (2014); Australia: Kraft (2009).

A monotypic genus. The type species was first described as *Myrionema expansum* by J. Agardh (1847) based on a sterile specimen and with very little information. Later, J. Agardh (1848) transferred this taxon to *Ralfsia*. *Ralfsia expansa* described by Weber-van Bosse (1913), Tanaka and Chihara (1980b) and Lim et al. (2007) based on materials from Indonesia, Japan and Malaysia/Japan, respectively were all characterised in having thallus with generally bilateral symmetry, distinct delineation of cortical and medullary layers, unilocular reproductive structures inserted on three to six-celled stalk and biseriate plurilocular reproductive structures with a sterile terminal cell (Fig. 2.13a). In contrast, Børgesen (1912; 1914) described *R. expansa* from the Danish West Indies with slightly different features: unilocular sporangia usually borne on a single-celled stalk, seldom stalkless; plurilocular sporangia biseriate, seemingly uncapped by a sterile cell (Fig. 2.13b). This situation is further complicated by the report of *R. expansa* from the Alboran Sea (Mediterranean Spain) with more or less bilateral medullary layer, unilocular sporangia sessile or on one-celled stalks and plurilocular sporangia with partly biseriate rows of loculi terminated with one dark coloured sterile cell (Sartoni and Boddi, 1989).

Ralfsia expansa sensu Tanaka & Chihara (1980b) shared a highly similar anatomy with *Ralfsia hancockii* (Dawson, 1944) from the Pacific coast of Mexico. The latter was described to have unilocular sporangia with a narrow basal stalk (illustrations included a detached paraphysis and a detached unilocular sporangium with at least three stalk cells) while plurilocular sporangia was not mentioned. According to León-Alvarez and González-González (2003) who examined the type specimen of *R. hancockii*, the thallus was mainly unilateral, cortical layer morphologically differentiated from the medulla and unilocular sporangia present on stalks of four to five cells. León-Alvarez and González-González (2003) considered *R. expansa sensu* Børgesen and *R. hancockii* to be distinct species and *R. expansa sensu* Tanaka & Chihara to be *R. hancockii*. In agreement with León-Alvarez and González-González (2003), I suspect that the specimens with unicellular stalk reported by Børgesen (1912) are probably limited to the Atlantic Ocean. Neither *R. expansa* sensu Tanaka & Chihara (1980b) nor *R. expansa* sensu Børgesen (1912) can be attributed to *R. expansa* (J. Agardh) J. Agardh unless one sequence the sterile holotype and Børgesen's morphotype for comparison. It was most unfortunate that the type specimen of *R. expansa* was sterile, which inadvertently lead to confusion as to which morphotype is the' real' *R. expansa*. Meanwhile, Lim et al. (2007) published *rbc*L sequences of *R. expansa sensu* Tanaka & Chihara and proposed the new genus *Neoralfsia* to accommodate the combination *N. expansa* which was shown to be molecularly distinct from several members of the genus *Ralfsia* including the generitype, *R. fungiformis*.

The genera *Neoralfsia* (*neo* - new) and *Ralfsia* share considerable similarities in anatomy and the former is primarily distinguished by its unilocular reproductive structures terminally inserted on three- to six-celled stalks, a single sterile cell terminating the plurilocular reproductive structures, apparent cortical and medullary layers and its vegetative filaments bilaterally curving up- and downward from the

central portion. Kraft (2009) suggested the place of collection to be a reliable indicator of *N. expansa* as opposed to true *Ralfsia* because very few other ralfsioid algae occur regularly in the tropics. According to Cormaci et al. (2012), the new combination *Neoralfsia expansa* (J. Agardh) Lim & Kawai by Lim et al. (2007) was invalid because they cited the wrong publication year of the original publication of the basionym.

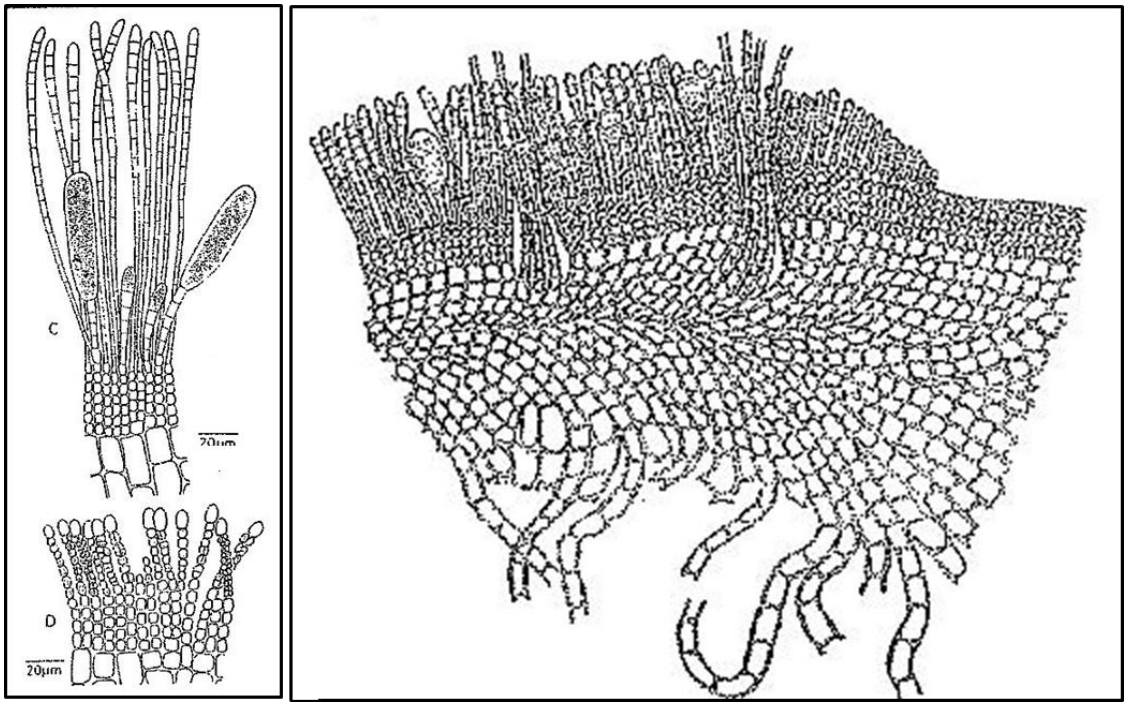

Figure 2.13a *Neoralfsia expansa*. Left: Unilocular reproductive structures (top) and plurilocular reproductive structures (bottom) [after Tanaka and Chihara 1980b, p. 230, fig. 2]. Right: Unilocular reproductive structures inserted among paraphyses on a bilaterally symmetrical thallus (after Weber-van Bosse 1913, p. 147, fig. 45).

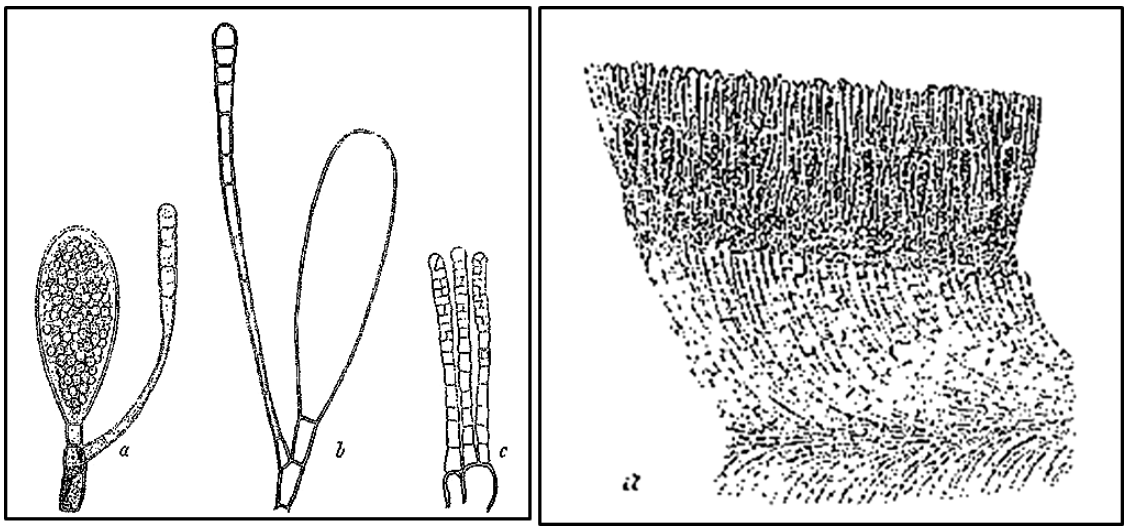

Figure 2.13b *Ralfsia expansa* sensu Børgesen. Left: Unilocular sporangium with a single stalk cell (labelled a and b) and plurilocular sporangia (labelled c). Right: Unilocular sporangia inserted among paraphyses on a bilaterally symmetrical thallus (after Børgesen 1912, pp. 124 - 125, figs. 1 - 2).

2.4.6 Other crustose brown algal genera associated with the Ralfsiaceae and/or Lithodermataceae.

2.4.6.1 *Diplura* Hollenberg (Fig. 2.14)

Generitype: *Diplura simulans* Hollenberg

Type locality: On rocks in the upper littoral zone, Corona del Mar, Orange County, California, North America

Distribution: North America (Abbott and Hollenberg, 1976); Japan (Tanaka and Chihara, 1981b); Hong Kong (Kaehler, 1994); New Zealand (Buchanan, 2005); Mexico (Pedroche et al., 2008); Australia (Womersley, 1987).

This genus was established by Hollenberg (1969) based on the type species from California and placed in the Ralfsiaceae. Members of this genus are characterised by a relatively thin crustose thallus, firmly adhering to the substratum without rhizoids, more or less gelatinous; erect filaments simple or sparsely branched and somewhat laterally adherent; multiple chloroplasts in each cell; plurilocular reproductive structures subterminal and capped by a single sterile cell; unilocular sporangia were unknown. Hollenberg (1969) remarked that the type species resembles *Mesospora* in its gelatinous, readily separated erect filaments but the two differed in their number of chloroplasts per cell (single in *Mesospora* and several in *Diplura*), structure of plurangia and the lack of unilocular sporangia for *Diplura*. Tanaka and Chihara (1981b) distinguished *Diplura* and *Endoplura* by the texture of their thalli (soft in *Diplura*, rigid in *Endoplura*) and the number of sterile cells terminating their plurilocular reproductive structures.

A second species, *D. simplex* was described by Tanaka and Chihara (1981b) from Japan who distinguished it from the type based on the size and thickness of thallus and the structure of erect filaments. Both Hollenberg (1969) and Tanaka and Chihara (1981b) conducted culture studies on their *Diplura* species in which cells liberated from the plurilocular reproductive structures gave rise to an expanded disc on germination, a diagnostic feature of the Ralfsiales. Buchanan (2005) reported a species from New Zealand which he tentatively named *Diplura* sp. "australis" and distinguished from other species of *Diplura* in having strongly laterally adherent upright filaments, in lacking hair pit and in being less gelatinous. Meanwhile, Womersley (1987) reported that one specimen of his collection from Southern Australia may be referred to *Diplura*. A molecular study by Lim et al. (2007) showed that *D. simplex* from Japan are distantly related to members of the Ralfsiales.

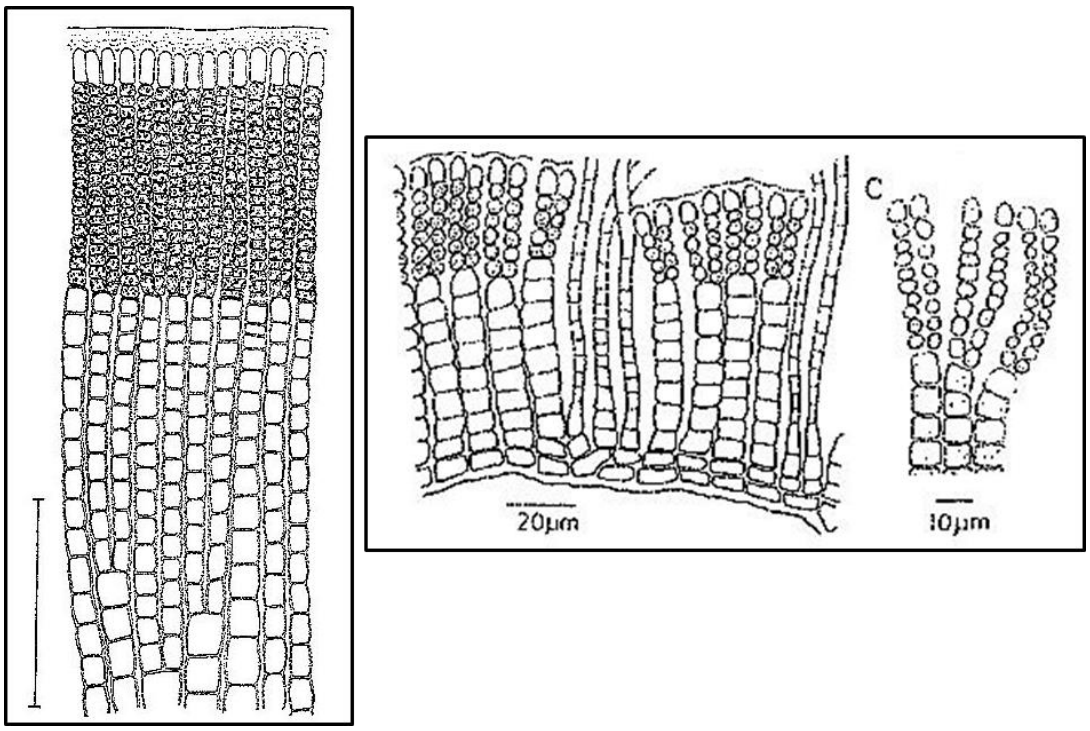

Figure 2.14 Left: *Diplura simulans* with plurangia (after Hollenberg 1969, p.299, fig. 25). Right: *D. simplex* shown with plurilocular reproductive structures and hairs (after Tanaka and Chihara 1981b, p. 156, fig. 1 B - C).

2.4.6.2 *Endoplura* Hollenberg

Generitype: *Endoplura aurea* Hollenberg (Fig. 2.15)

Type locality: A shallow high tide pool on a large flat rocky point near the foot of Fairwiew Place, Laguna Beach, Orange County, California.

Distribution: Pacific Ocean - North America (Abbott and Hollenberg, 1976), Mexico (Pedroche et al., 2008), Japan (Tanaka and Chihara, 1981b), Hong Kong (Kaehler, 1994).

A monotypic genus, placed in the Ralfsiaceae (Hollenberg, 1969) on account of its intercalary plurangia. According to Hollenberg (1969), the distinctive features of this genus are in its submerged position of the plurangia and the numerous chloroplasts per

cell. The type species was also described to have thallus forming golden (hence, the specific epithet) to light brown crusts, firmly attached to the substratum without rhizoids, not gelatinous, erect filaments firmly adjoined and infrequently branched, arising from an indistinct hypothallial layer; plurangia in irregular sori, initially uniseriate but ultimately biseriate to quadriseriate, with two to three (to five) terminal sterile cells (consistently three in Japanese materials). Unilocular sporangia are unknown. Tanaka and Chihara (1981b) conducted a culture study on this alga and observed that cells liberated from the plurilocular reproductive structures germinated and developed into an expanded disc. A molecular study by Lim et al. (2007) inclusive of Japanese material of *E. aurea* indicated that this species belonged to the Ralfsiaceae in the order Ralfsiales.

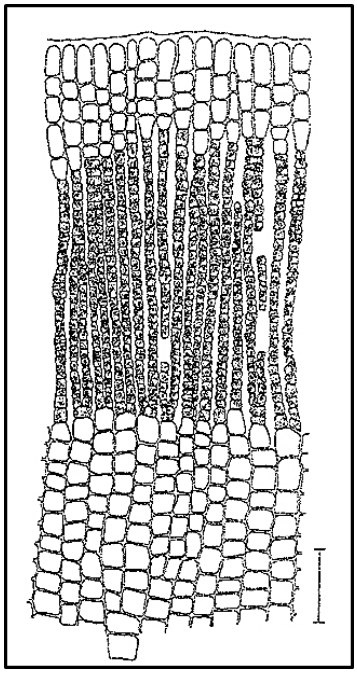

Figure 2.15 *Endoplura aurea* with plurangia (after Hollenberg 1969, p. 299, fig. 24)

2.4.6.3 *Zeacarpa* Anderson, Simons & Bolton

Generitype: *Zeacarpa leiomorpha* Anderson, Simons & Bolton (Fig. 216)

Type locality: Hout Bay, Cape Peninsula, South Africa

Distribution: South-western Cape coast, South Africa

A monotypic genus. This genus was established by Anderson et al. (1988) based on material from southern Africa and placed in the Ralfsiaceae. The genus name is derived from the superficial resemblance of the unilocular sporangia to cobs of maize while the specific epithet of the type refers to the smooth surface of the crust (*leios* meaning smooth in Greek). *Zeacarpa* is characterised by a smooth crust tightly adherent to the substratum; thallus consisting of little branched vegetative filaments up to 30 cells long, closely coherent even under pressure; cells each with several chloroplasts without pyrenoids; sorus consisting of free, mucilaginous, uniseriate, unbranched and distally tapering filaments; one to three intercalary sporangiferous cells in each fertile soral filament, each giving rise to, laterally, and in succession, up to ten unilocular sporangia; unilocular sporangia fusiform or acerose in shape, containing 16 spores when mature; plurilocular sporangia were unknown. The lateral origin of its unilocular sporangia is a distinctive character for this genus among the other crustose brown algal genera. The term ‘soral filaments’ was used in place of ‘paraphyses’ as the latter are by definition, sterile filaments and thus, inapplicable to *Zeacarpa*. Anderson et al. (1988) reported that spores from unilocular sporangia germinated without a sexual fusion and underwent a discoid type early development.

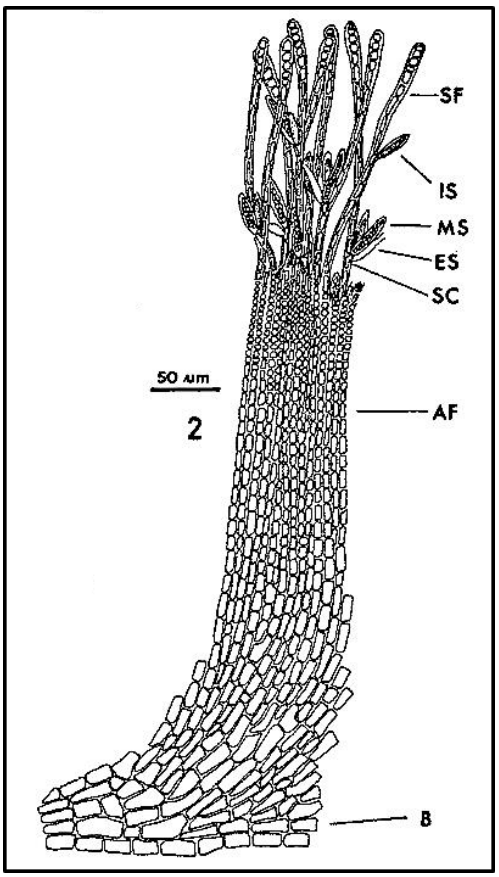

Figure 2.16 *Zeacarpa leiomorpha* with soral filaments (SF) comprising sporangiferous cells (SC) bearing immature sporangia (IS), a mature sporangium (MS), an empty sporangium (ES), the basal layer (B) and ascending filaments (AF). [After Anderson et al. 1988, p. 322, fig. 2].

2.4.6.4 *Petroderma* Kuckuck

Generitype: *Petroderma maculiforme* (Wollny) Kuckuck (Fig. 2.17)

Type locality: Helgoland, Germany

Distribution: Described as a cold water alga, commonly found in the northern Atlantic Ocean, less frequent in the Pacific Ocean - British Isles (Fletcher, 1978); Norway (Jaasund, 1965); Sweden (Waern, 1949); Iceland (Jónsson, 1903); Greenland (Wilce et al., 1970); South Georgia (Skottsberg, 1921); North America (Edelstein and McLachlan, 1968; Wilce et al., 1970); Red Sea (Rayss and Dor, 1963).

Kuckuck (1897) transfered *Lithoderma maculiforme* Wollny to *Petroderma*, a new genus he established on the basis of its small crusts; erect filaments mostly unbranched, closely packed like *Lithoderma* but easily separated under pressure, gelatinous, arising from a monostromatic basal layer; each cell consists a single chloroplast; terminal unilocular sporangia oblong-oval and inconspicuous, at times occurring in small clusters at the apex of a single filament; plurilocular sporangia (less frequently reported) terminal on erect filaments, variable, some long and uniseriate while others may be short, multiseriate and branched, sometimes occurring in the same sorus as unilocular sporangia. The description and illustrations by Wollny (1881) were brief and rather uninformative apart from the fact that *L. maculiforme* possess unilocular sporangia, with structure similar to *Lithoderma*, marine in nature and form small and soft crusts; in addition the same author suspected this alga to be identical with *L. fluviatile* but decided against it as the latter was freshwater in habit. Edelstein and McLachlan (1968), Fletcher (1987) and Sanders et al. (2005) observed collar-like remnants of old sporangial walls along the filaments, which were proposed as a generic feature of *Petroderma* by Waern (1949). Apart from the type species, two other species included in this genus are *P. steinitzii* Rayss & Dor (1963) reported from the Red Sea, Israel and *P. vietnamensis* Pham-Hoàng Hô (1969) reported from Vietnam. Wynne (1969) and Wilce et al. (1970) disagreed with the inclusion of *P. steinitzii* in *Petroderma* by pointing out Rayss and Dor's (1963) inconsistent report of plastid number and the character of the sorus. Wilce et al. (1970) went further by suggesting that *P. steinitzii* to be included in another genus such as *Myrionema*.

Kuckuck (1897) distinguished *Petroderma* from *Lithoderma* based on the number of plastids per cell and the structure of reproductive organs. *Petroderma* and *Symphyocarpus* shared similarities in their terminal multiseriate plurilocular sporangia, loosely united erect filaments, number of plastid per cell and reports of highly

pigmented ascocyst-like structures. Kjellman and Svedelius (1910) included *Petroderma* in the Lithodermataceae and distinguished this genus from *Sorapion*, *Lithoderma* and *Pseudolithoderma* by the association of erect filaments (loosely versus closely adherent) and from *Heribaudiella* by the reproductive sori (combined versus scattered). Fletcher (1978) described the generitype with fairly soft crust, gelatinous and near sponge-like in appearance when fully grown, constructed of loosely bound erect filaments and that the single plate-like plastid is frequently lobed in appearance and in surface view may be mistaken for discrete discoid plastids. Short multicellular rhizoids and/or hairs terminating erect filaments were sometimes reported (Edelstein and McLachlan, 1968; Fletcher, 1987; Peňa and Bárbara, 2010); nonetheless hairs were not observed by Kuckuck (1897). Highly pigmented ascocyst-like structures have also been reported in *P. maculiforme* (Kuckuck, 1897; Skottsberg, 1921; Fletcher, 1987; Peňa and Bárbara, 2010). Waern (1952), Wilce et al. (1970) and Fletcher (1987) considered *Lithoderma lignicola* Kjellman to be synonymous with *P. maculiforme* but such conclusion is questionable (Edelstein and McLachlan, 1968) since Kjellman's specimen was sterile. Fletcher (1974, 1978) reported that a direct life history with the absence of a sexual phase was observed (via field observations and culture studies) for the type species which was also noted to possess only a crustose phase, suggesting that the separate plurilocular sporangiate thalli were not gametophytic in origin; in addition unispores were likely to develop apomeiotically. Culture studies by Wynne (1969) also indicated a filamentous development in *P. maculiforme*. This species has also been reported as a phycobiont of the lichen fungus, *Verrucaria tavaresiae* Moe and appeared to be the only brown alga playing such a role (Sanders et al., 2005). Conflicting reports concerning its possession of a pyrenoid were noted in which light microscope observations of *P. maculiforme* did not show presence of pyrenoid in most studies (Wilce et al., 1970; Fletcher, 1987) but a TEM study by Sanders et al. (2005) detected a

pyrenoid in this alga which the authors suggest was left out in previous reports due to limitations in light microscopy rather than being absent in the populations examined. The generitype was reported to tolerate a broad range of salinities; growing both lithophytically and epiphytically; and does not tolerate long periods of dessication and strong wave action (Wilce et al., 1970). In a molecular study by Bittner et al. (2008), *P. maculiforme* was resolved near the basal portion of the tree, as a sister to *Ishige okamurae* Yendo and the authors suggested establishment of the order Petrodermatales to accommodate this taxon. However, the data concerning this new order has yet to be published.

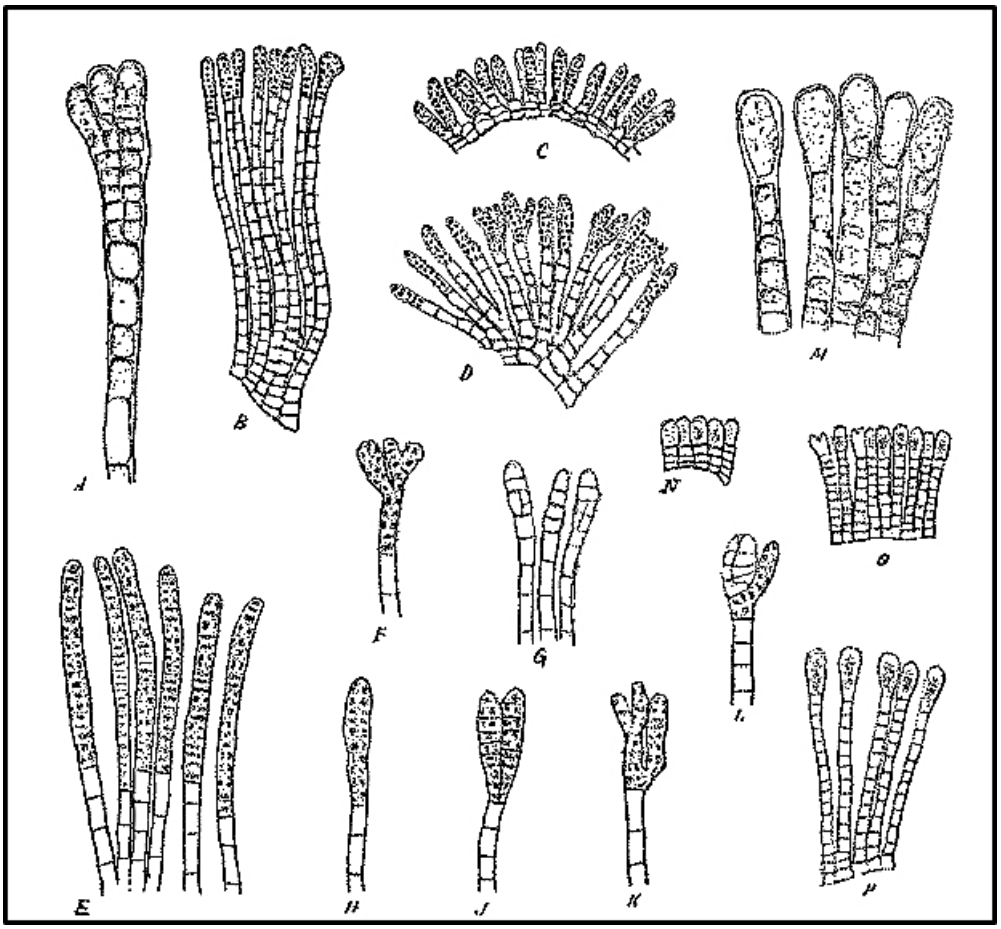

Figure 2.17 *Petroderma maculiforme.* A - L: Plurilocular sporangia. M - P: Unilocular sporangia. Emptied sporangia are shown in G and L. (after Kuckuck 1897, p. 384, fig. 10)

2.4.6.5 *Porterinema* Waern (Fig. 2.18)

Generitype: *Porterinema fluviatile* (Porter) Waern

Type locality: Unter-Warnow between Rostock and Warnemünde, Germany

Distribution: North America (Wilce et al., 1970; Wehr and Perrone, 2003); Europe (Sweden: Waern, 1952; Norway: Jaasund, 1965; Netherlands: Dop, 1979; Germany: McCauley and Wehr, 2007)

This genus was founded by Waern (1952) based on its peculiar plurilocular sporangia and placed in the Lithodermataceae based on its possession of terminal unilocular sporangia resembling those of *Lithoderma* and *Sorapion*. The type species was first described as *Streblonema fluviatile* (Porter, 1894) with creeping, branched, free to almost pseudoparenchymatous filaments; one to several plastids and four-chambered sporangia. The diagnostic features of *Porterinema* include microscopic, endophytic, epiphytic or epilithic thallus; the prostrate portion consists of vegetative branching filaments forming an orbicular layer; the erect portion comprises sporangia and hair; plurilocular sporangia intercalary, sessile or pedicellate (sometimes shaped like a crown), each loculus dehisced through individual apical opening; unilocular sporangia (rare) elongated, in sorus. Waern (1952) observed in *P. fluviatile* one to two parietal disc-like plastids (probably depending on age as suggested by Wilce et al., 1970) and direct germination of swarmers from plurilocular sporangia without fusion. *Porterinema* is described to be a euryhaline and uncommon genus; its vegetative thallus is easily confused with species of other genera such as *Streblonema*, *Hecatonema*, *Phaeostroma* and *Endodictyon* (Wilce et al., 1970).

Two species have been attributed to this genus i.e. *P. fluviatile* (Fig. 2.18a) and *P. marina* (Fig. 2.18b). The latter was established by Jaasund (1965) who distinguished

this from the type species based on its more uniform size and the marine character of its habitat. Wilce et al. (1970) consider both species to be conspecific; a comparison of the filament width between different populations of *Porterinema* from literatures, indicated that the size range reported by Jaasund (1965) for *P. marina* falls within the size range given by other authors for *P. fluviatile*. In addition, these authors found that *Porterinema* populations collected from brackish water developed plurilocular organs in high salinity culture solutions, further supporting the synonymy of the two species. *Petroderma* and *Porterinema* can be differentiated through their manner of crust development and the position of reproductive structures (Wilce et al., 1970). The *Porterinema* crust consists mostly of prostrate filaments, loosely anastomosing and highly branched while the erect filaments are only a few cells in length. Meanwhile the *Petroderma* crust is made up of sparsely branched erect filaments of five to 35 cells in length arising from a monostromatic layer. Both types of reproductive structures are variously inserted and clustered along a filament in *Porterinema* and terminally inserted in *Petroderma*. Although the systematic position of *Porterinema* is currently uncertain, a molecular study by McCauley and Wehr (2007) demonstrated the exclusion of *P. fluviatile* in the Ectocarpales. Tanaka and Chihara (1981c) suggested that *Porterinema* be excluded from the Lithodermataceae and probably from the Ralfsiales on the basis of its peculiar plurilocular bodies and thalli construction which is composed of only creeping filaments attached to substratum and lack clear erect filaments. Furthermore, Draisma et al. (2010) in his study using the *rbc*L and *psb*C gene suggested the early divergence of the *Porterinema* within the Phaeophyceae because of the 12 nucleotide gap in its *psb*C sequence. He expected this gap to occur in other basal taxa such as *Ishige*, *Diplura*, *Petroderma* and sister classes of the Phaeophyceae. According to Guiry and Guiry (2013), the genus *Pseudobodanella* Gerloff is currently treated as a synonym of *Porterinema*.

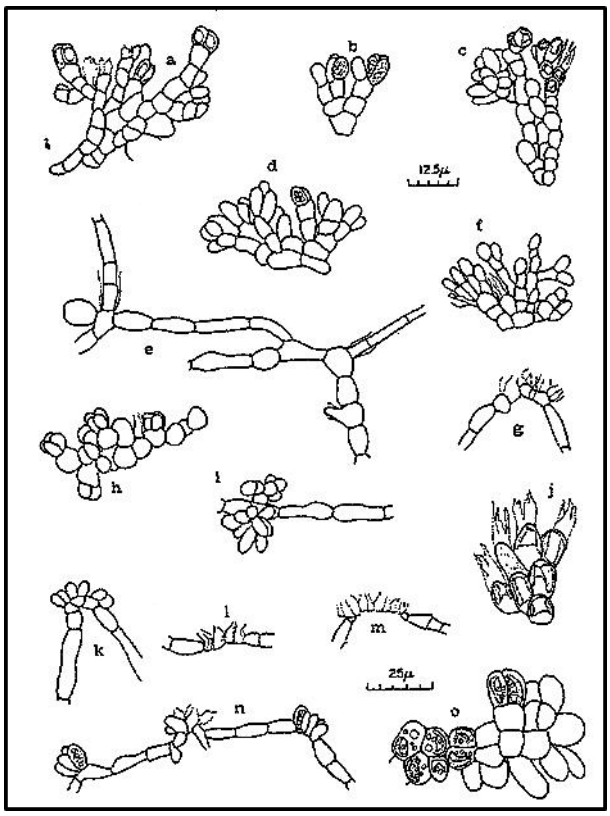

Figure 2.18a *Porterinema fluviatile* showing variation in the insertion of four-parted reproductive structures: sessile (g, h, k), intercalary (l - n) and pedicellate (a - b, f, i); sheathed hair is shown in e, and possibly the unilocular sporangia of this species in j and o (after Wilce et al. 1970, p. 131, fig. 4).

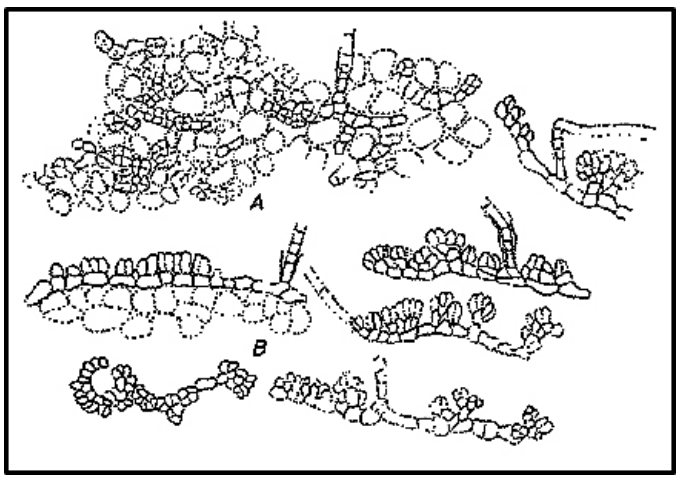

Figure 2.18b *Porterinema marina* in *Dictyosiphon chordaria*. A: seen from above. B: in cross sectional view. Dotted lines mark the host tissue. (after Jaasund 1965, p. 46, fig. 13 A - B).

2.4.6.6 *Sorapion* Kuckuck

Generitype: *Sorapion simulans* Kuckuck (Fig. 2.19)

Type locality: Helgoland, south-eastern corner of North Sea

Distribution: Britain (Fletcher, 1987); Ireland (Guiry, 2012); Baltic Sea (Nielsen et al., 1995); East Greenland (Lund, 1959); Canadian Arctic (Lee, 1980); Scandinavia (Athanasiadis, 1996).

Kuckuck (1894) established the genus *Sorapion* on the basis of *S. simulans* from Helgoland. The diagnostic features of this genus include thallus with a monostromatic basal plate from which erect filaments arise, a single plate-shaped chloroplast per cell, terminal pear-shaped unilocular sporangia in small separate sori and plurilocular sporangia were unknown. Pyrenoid was absent in Kuckuck's (1894) report on *S. simulans*, however it was present in British materials as reported by Fletcher (1987). The type species tested negative in the diagnostic test using sodium hypochloride for inclusion in the Sphacelariales (Kuckuck, 1894). Both Kuckuck (1894) and Hamel (1935) distinguished *Sorapion* from *Lithoderma* based on the number of chloroplasts per cell (single vs. several), shape of sporangia (pear shaped vs. oval) and the structure of sporangial sori (small scattered vs. large indefinite patch). *Sorapion* is generally distinguished from *Petroderma* based on the organisation of erect filaments (loosely adjoined in *Petroderma*, more tightly adherent in *Sorapion*) and the absence of plurilocular sporangia in *Sorapion*. Kjellman and Svedelius (1910) distinguished *Sorapion* from *Pseudolithoderma* based on the number of chloroplasts per cell, the shape of unilocular sporangia and the structure of sporangial sori. Batters (1896) transferred *S. simulans* to *Lithoderma*, forming the combination *Lithoderma simulans*

(Kuckuck) Batters as he considers the characteristics for delineation of the two genera insufficient but Kuckuck (1897) rejected this.

Sorapion kjellmanii is the only other species of this genus and it was reported by Lee (1980) to be epilithic, epizoic on invertebrates or epiphytic on other algae. Lund (1959) described his *S. kjellmanii* to have cells harbouring a plate-like plastid with a visible pyrenoid and presence of phaeophycean hair. This species was first described by Wille (in Wille and Rosenvinge, 1885) as *Lithoderma kjellmanii*, growing on *Chaetomorpha melagonium* (Weber & Mohr) Kützing. Rosenvinge (1899) transferred *L. kjellmanii* to *Sorapion* on the basis of the single chloroplast per cell feature which is consistent with the type species, and even went further by suggesting the two to be a single species as he considers the difference in structure of sporangial sori to be insufficient for species delimitation although the author did acknowledge the difference in pyrenoid presence/absence between the two taxa. The (minor) differences between *S. simulans* and *S. kjellmanii* in which the latter lacks pyrenoid (contrary to the observation by Rosenvinge and Lund), filaments branched towards the apex, less well defined sporangial sori and presence of hair again lead Fletcher (1987) to suggest their conspecificity. Similarly, *Sorapion simulans* was also linked as an alternate phase in the life-history of *Porterinema fluviatile* (Pedersen, 1981b). Sears and Wilce (1973) indicated that terminal plurilocular sporangia are present in *Sorapion*.

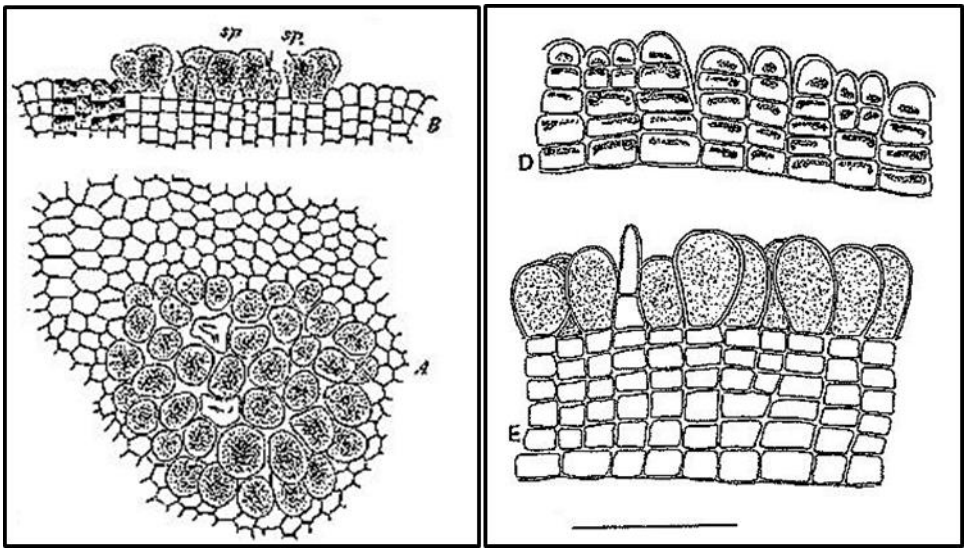

Figure 2.19 *Sorapion simulans* Left: A sorus of unilocular sporangia A. surface view B. vertical section (after Kuckuck 1894, p. 237, fig. 10). Right: D. Vertical section of vegetative crust showing cells with a single, large, plate-like chloroplast with occasional pyrenoid. E. Vertical section of fertile crust showing large, terminal pyriform, unilocular sporangia. Bar = 50μm (after Fletcher 1987, p. 89, figs. 4D - E).

2.4.6.7 *Symphyocarpus* Rosenvinge

Generitype: *Symphyocarpus strangulans* Rosenvinge (Fig. 2.20)

Type locality: Godhavn, west coast of Greenland

Distribution: Cold waters of the subpolar and temperate region - Canadian Arctic (Lee, 1980); Greenland (Lund, 1959), Britain and Ireland (Fletcher, 1987); Scandinavia (Athanasiadis, 1996); Spain (Bárbara et al., 2004); Baltic Sea (Nielsen et al., 1995); North Norway (Jaasund, 1965)

Rosenvinge (1893) established this genus and placed it under the Ectocarpaceae but Kuckuck (1894) disagreed on the basis of the sporangium and placed this genus under the Scytosiphonaceae. Considering that both families are now placed in the order Ectocarpales *sensu lato* and in addition to *Symphyocarpus*'s similarity to

Microspongium, there is a high possibility of this genus belonging to that order. This genus is characterised by a monostromatic basal layer composed of branched, creeping filaments, initially free later coalescing to form a parenchyma; erect filaments short [2 - 7 (-11) cells], simple or subdichotomously branched, loosely united and slightly gelatinous; cells containing a single disc-shaped chloroplast; terminal and multiseriate plurilocular sporangia and unilocular sporangia were unknown. Lund (1959) observed in *S. strangulans* of plurilocular sporangia which were more elongated and reported that a vertical filament may bear a terminal bundle of not less than 8 sporangia, with the formation of spores being basipetal. Kuckuck (1894) reported the presence of a large pyrenoid at the side of the chloroplast. Rosenvinge (1899) described inflated terminal cells of erect filaments containing dense yellowish pigments which Fletcher (1987) and Bárbara et al. (2004) termed as ascocysts. According to Rosenvinge (1893), the type species was epiphytic on *Chaetomorpha melagonium* and the epiphytic habit was also reported by Jaasund (1965) and later, Fletcher (1978). Meanwhile, it was found on subtidal maërl and gravel bed at the northwest Iberian Peninsula by Bárbara et al. (2004) and epizoic on dogfish cases by Fletcher (1987).

The other species of *Symphyocarpus*, *S. longisetus* Lund (Fig. 2.21) is described as an epiphytic, small cuhion-shaped, solid alga with a firm structure containing one (or two) small disc-shaped or irregularly lobed plastid(s), apparently with a pyrenoid and possess hairs. Lund (1959) distinguished this species from the generitype by the long basal cell of its hairs, appearance and shape of the cells in the erect filaments (hyaline and constricted at the transverse walls) and the absence of ascocyst-like cells. Although Lund (1959) highlighted the similarity of the long basal cell of hairs between his alga and the type species of *Phaeostroma*, he dismissed its taxonomical importance and instead placed the alga in *Symphyocarpus*. Nevertheless, this species is currently treated as a taxonomic synonym of *Phaeostroma longisetum* (Lund) Pedersen. Pedersen

(1981a) conducted a culture study on *S. longisetus* and found that it agrees with the type species of *Phaeostroma* with regard to its mode of formation of plurilocular sporangia, number of chloroplasts, and type of true hairs, and disagrees with the type species of *Symphyocarpus*.

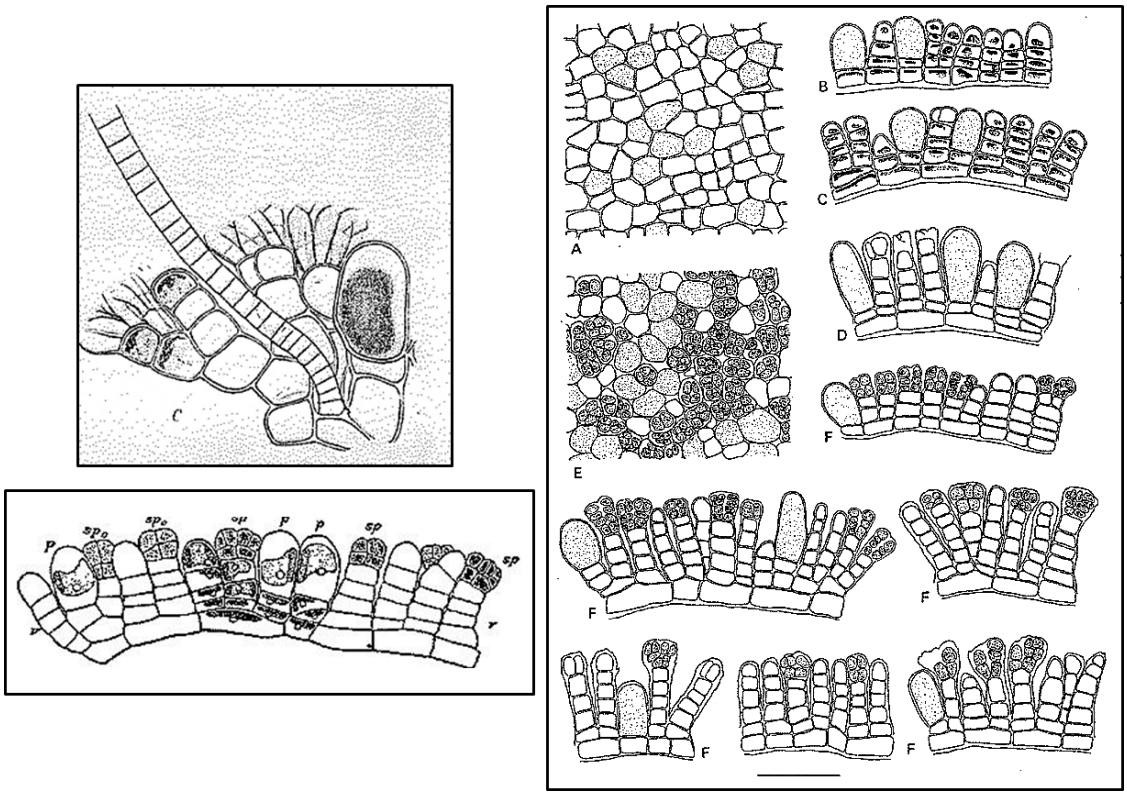

Figure 2.20 *Symphyocarpus strangulans*. Top left: VS through a thallus with depleted plurilocular sporangia, to the right, a larger cell with brown refractive contents is seen (after Rosenvinge 1893, p. 897, fig. 29). Bottom left: Squashed preparation showing *v* vegetative cells, *sp* mature plurilocular sporangia, sp_o young plurilocular sporangia, *p* enlarged terminal cell, plate-form chloroplast with large pyrenoid is also shown (after Kuckuck 1894, p. 236, fig. 9). Right: A. Surface view of vegetative crust showing scattered, hyaline ascocysts. B - D. VS of vegetative crusts showing erect filaments, large, hyaline ascocysts and cells with a single plate-like chloroplast. E. Surface view of fertile crust showing ascocysts and plurilocular sporangia with 2 - 4 loculi. F. VS of fertile crusts showing erect filaments, ascocysts and terminal plurilocular sporangia (after Fletcher 1987, p. 91, fig. 5).

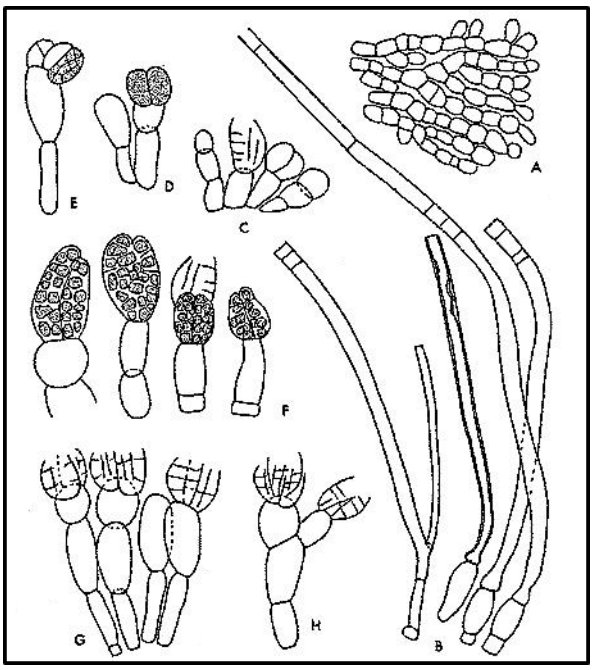

Figure 2.21 *Symphyocarpus longisetus* showing young, mature and emptied plurilocular sporangia (after Lund 1959, p. 75, fig. 13).

2.4.6.8 *Acrospongium* Schiffner

Generitype: *Acrospongium ralfsioides* Schiffner (Fig. 2.22)

Type locality: On the small island of Banjol, Rovigno, Croatia (Mediterranean and Adriatic Sea)

Distribution: Europe-Mediterranean (Sartoni and Boddi, 1989; Ribera et al., 1992)

A monotypic genus. This genus is characterised by fairly soft texture of the crust; free, mucilaginous, simple or mostly unbranched erect filaments arising from a laterally adherent basal layer of three to seven cells thick; unilocular sporangia terminally inserted on short filaments, at times intercalary but never lateral; plurilocular sporangia were unknown and single parietal plate-like plastid per cell without obvious pyrenoid. Schiffner (1916) found the specimens growing on sterile crusts of *Lithoderma*

adriaticum on a stone. The author distinguished this genus from a) the closely related *Microspongium* by the multilayer basal plate and the terminal (not lateral) unilocular sporangia; and b) *Ralfsia* by the absence of rhizoids and terminal sporangia. Sartoni and Boddi (1989) described *A. ralfsiodes* from the Alboran Sea with erect filaments of 12 - 16 cells; unilocular sporangia common, without paraphyses, borne terminally on erect filaments either close to the basal layer or with a few-celled stalk. They also reported the occasional occurrence of intercalary unilocular sporangia, arising from a cylindrical cell in the lower half of the erect filament which gradually enlarges, displacing to one side the distal position of the filament. A micrograph of the squash preparation of *A. ralfsiodes* by Sartoni and Boddi (1989, p. 147, fig. 1) bore a striking resemblance to *Mesospora*. In the same study, *Acrospongium* was compared to *Mesospora*, *Hapalospongidion* and *Basispora* on account of its unilocular sporangia without paraphyses borne terminally on short, erect filaments. They pointed out the presence of a single plate-like plastid as a similarity between *Acrospongium* and *Mesospora*; while *Acrospongium* and *Hapalospongidion* both possess unilocular sporangia borne terminally on ordinary vegetative filaments whereas in *Mesospora* and *Basispora*, the unilocular structures grow on specialised stalks. In addition, Sartoni and Boddi (1989) suggested a possibility of *A. ralfsiodes* being a heteromorphic phase of the Scytosiphonaceae due to the lack of plurilocular sporangia and the morphological plasticity occasionally observed in the erect filaments. The authors were careful in pointing out the provisional placement of this taxon in the Ralfsiaceae pending more information of the life history from culture studies. Buchanan (2005) suggested the combination of this genus with *Hapalospongidion*.

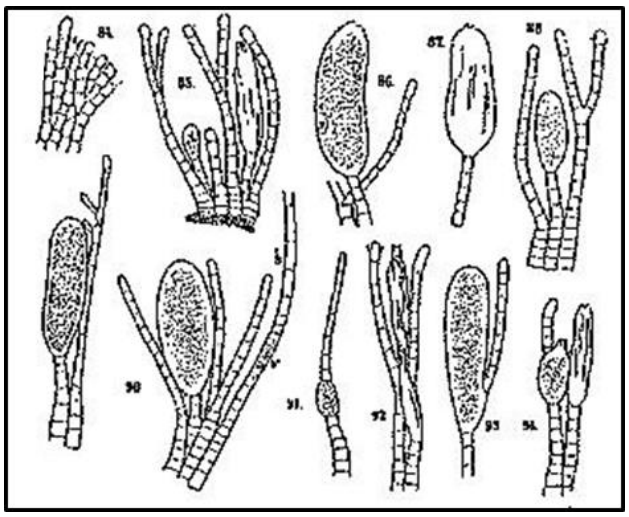

Figure 2.22 *Acrospongium ralfsioides* with unilocular sporangia at terminal (labelled 86 - 89) and intercalary (labelled 91 - 94) positions. (after Schiffner 1916, p. 158, figs. 84 - 94).

2.4.6.9 *Heribaudiella* Gomont

Holotype: *Heribaudiella arvernensis* Gomont

Taxonomic synonym and currently accepted name for type species: *Heribaudiella fluviatilis* (Areschoug) Svedelius (Fig. 2.23)

Type locality: near the village of Molompize, near Massiac, Haute-Auvergne, France

Distribution: North America (Wehr and Perrone, 2003), Europe (Bulgaria: Stoyneva et al., 2003; Germany: McCauley and Wehr, 2007; France: Eloranta et al., 2011; Britain: Wehr, 2002; Sweden: Areschoug, 1875), Asia (Japan: Kumano and Hirose, 1959; China: Jao, 1941; Hu and Wei, 2006).

This genus is one of the few brown algal genera living in freshwater environment and described by Svedelius (1930) as the 'freshwater *Lithoderma*'. The type species is typically found colonizing rocks in clear, rapidly flowing water (Wehr and Perrone,

2003). Gomont (1896) established the genus *Heribaudiella* (named after Frère Joseph Heribaud, a French diatomist and plant collector) on account of the holotype, *H. arvernensis* and placed it in the Ralfsiaceae. Gomont (1896) who based his description on dried specimens, described *H. arvernensis* as thin (less than 200 μm thick) and expanded with indefinite outline; erect filaments encompassed in gelatinous material and united only by their basal layer, thus easily separated by pressure (distinguished from *Lithoderma*); dichotomous and lateral branching which gave rise to a fastigiate appearance; terminal unilocular sporangia not gathered in a sorus; plurilocular sporangia unknown and seemingly single chloroplast per cell. The same author also highlighted the similarity of this genus with *Symphyocarpus* in terms of their thalli construction and free filaments but added that erect filaments usually branched with false dichotomy and that the basal layer was not tightly coalescent in *Symphyocarpus*. The name *H. arvernensis* is currently regarded as a taxonomic synonym of *Heribaudiella fluviatilis* whose basionym is *Lithoderma fluviatile* Areschoug (1875). Wollny (1886) described and illustrated (Tab. I., fig. 3) a structure which he deduced to be the antheridia of *L. fluviatile*.

Svedelius (1930) provided a revised diagnosis of the genus *Heribaudiella* which include thallus crustose; the entire underside adherent to the substratum; monostromatic basal layer from which dichotomously branched and laterally free erect filaments arise; chloroplasts several, disc-shaped, rounded or polygonal; unilocular sporangia arise from transformation of apical cells; plurilocular sporangia arise from transformation of a series of apical cells, consisting of a single row of locules that do not differ significantly from vegetative cells; sporangia not aggregated in sori; and both types of reproductive structures sometimes found in the same crust. In the same study, the author brought together *L. fluviatile*, *L. fontanum* Flahault and *H. arvernensis* under the name *H. fluviatilis* (Areschoug) Svedelius. However, Hamel (1935) considered *L. fontanum* to be

a more developed form of *L. fluviatile* and indicated the possibility of *H. arvernensis* as a distinct species on the basis of its alleged single chloroplast per cell (needs to be verified with living materials) and more easily separable filaments. Flahault (1883) described *L. fontanum* from Montpellier (France) and distinguished it from *L. fluviatile* (first described from Sweden) based on the former's larger crust and thalli comprising a larger number of cells of the erect filament. Wollny (1886) also mentioned that *L. fontanum* is very different from *L. fluviatile*. Meanwhile, Eloranta et al. (2011) described *Lithoderma zonata* C.-C. Jao from China as a synonym of *H. fluviatilis*. Kjellman and Svedelius (1910) included *Heribaudiella* in the Lithodermataceae and distinguished *Heribaudiella* from other members of this family: a) *Petroderma* on account of their reproductive structures (the former scattered, the latter combined) and from b) *Sorapion*, *Pseudolithoderma* and *Lithoderma* based on the cohesiveness of their erect filaments. The systematic position of this genus at the familial and ordinal levels is still unclear (generally labelled as *incertae sedis*). Although a molecular study by McCauley and Wehr (2007) indicated a close relationship between *H. fluviatilis* and the Sphacelariales, their study also demonstrated that this alga tested negative in the diagnostic test for members of Sphacelariales using sodium hypochloride. In addition, the same authors found that *H. fluviatilis* is not monophyletic whereby *H. fluviatilis* from Canada was not identical with *H. fluviatilis* from Germany but sister to *Bodanella lauterborni* Zimmermann.

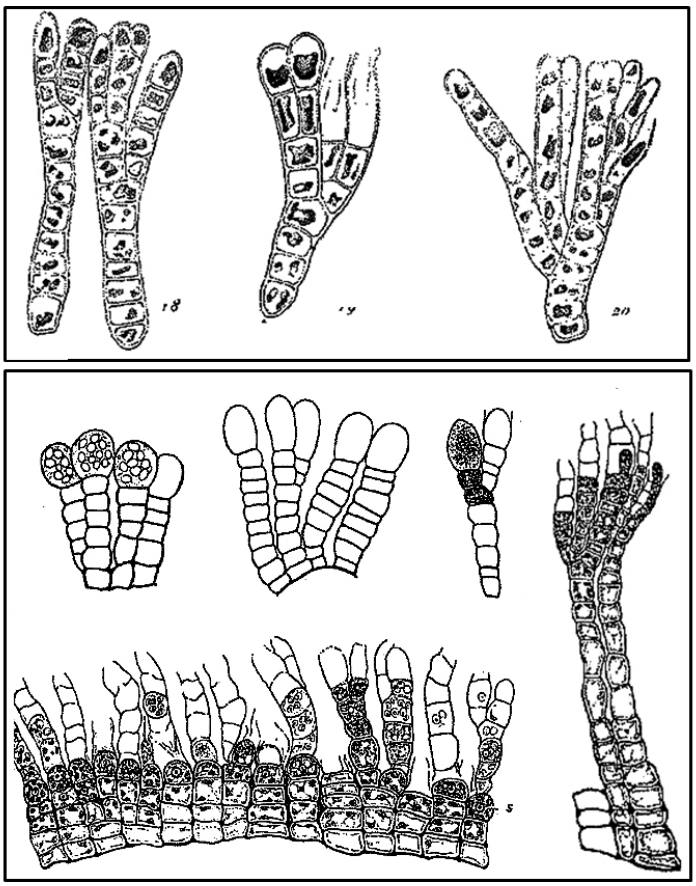

Figure 2.23 *Heribaudiella fluviatilis*. Top: terminal unilocular sporangia and dichotomously branched erect filaments (after Gomont 1896, figs. 17-20, pl. X); bottom: unilocular sporangia and plurilocular sporangia (after Svedelius 1930; figs 5, 6, 8, 11; pp. 904, 905, 907)

2.4.6.10 *Analipus* Kjellman (Fig 2.24)

Holotype: *Analipus fusiformis* Kjellman

Taxonomic synonym and currently accepted name for type species: *Analipus filiformis* (Ruprecht) Papenfuss

Type locality: Bering Island (Russia)

Distribution: Generally found in the cold water region of North Pacific Ocean - Japan (Okamura, 1936; Kogame et al., 1998); North America (Abbott and Hollenberg, 1976); Russia (Titlyanov and Titlyanov, 2012); Korea (Lee and Kang, 2001).

This genus is a unique member of the Ralfsiales with both the gametophyte and sporophyte stages in erect form. It was established by Kjellman (1889) based on the type species, *A. fusiformis* and was placed in the family Chordariaceae. In 1967, Papenfuss transferred *Haplosiphon filiformis* Ruprecht (1850) to *Analipus*, hence the combination *A. filiformis* (Ruprecht) Papenfuss which is a heterotypic synonym of the holotype. Two other species currently accepted taxonomically are *A. japonicus* (Harvey) Wynne and *A. gunjii* (Yendo) Kogame & Yoshida. Wynne (1971) merged *Heterochordaria* Setchell & Gardner into *Analipus* which he placed in the Chordariaceae, Chordariales (doing away with Heterochordariaceae) as he considers the trait of presence (*Heterochordaria abietina* (Ruprecht ex Farlow) Setchell & Gardner, the generitype of *Heterochordaria*) or absence (*A. filiformis*) of laterals on the erect axes insufficient for generic distinction. Consequently, the new combination *A. japonicus* (Harvey) Wynne was made for *Heterochordaria abietina* in the same study. *A. japonicus* is distinguished from *A. filiformis* by its possession of numerous lateral branchlets on the erect axes and longer erect axes [2 - 8 (to 12) cm in *A. filiformis*; 20 - 25 cm in *A. japonicus*] whereas *A. gunjii* is distinguished from these two species based on its discoid basal system which differs from the branched basal system in the other two species. Presence of branchlets is rare, sometimes with protuberances in *A. gunjii* (Kogame et al., 1998).

Analipus is defined by having erect axes arising from a well-developed perennial prostrate rhizomatous basal portion. The basal system consists of densely branched, tuberous and lobed intergrown filaments forming a crust-form complex while the erect

axes consist of cylindrical branches composed of cells with thick cell wall and of yellowish-brown phenolic materials. The crust and upright axes are not separate life history phases but differentiated parts of the same phase (Nelson, 1982). The erect axes are 1 - 3 mm in width; hollow in the upper portion and solid in the lower portion; medullary layer composed of elongated cylindrical cells whereas cortical layer consist of assimilatory filaments. Each cell contains a single parietal cup-shaped chloroplast without pyrenoid and a lot of physodes, exceptionally inner cells contain several discoidal chloroplasts. Both unilocular and plurilocular sporangia are found on the erect axes of separate thalli. Plurilocular structures uni- or more frequently biseriate, arise from transformation of the assimilatory filaments with one to three sterile terminal cells. Unilocular sporangia arise from the basal parts of clavate assimilatory filaments consisting of four to five cells each, with an enlarged terminal cell. In a study by Nelson and Cole (1981) to measure the relative DNA content of nuclei in *A. japonicus*, it was found that this species exhibits caryologically distinct isomorphic phases; unispores and plants bearing plurilocular structures are haploid while plants bearing unilocular sporangia are diploid and meiosis takes place within the unilocular sporangia prior to the formation of unispores. Kogame et al. (1998) reported *A. gunjii* from Hokkaido to be dioecious, produced weakly anisogamous gametes and occurence of gamete fusion. Nakamura (1972) placed *Analipus* in the Ralfsiaceae on the basis of its discal-type development and its reproductive structures, and this was supported by Wynne (1972). A molecular study by Lim et al. (2007) showed that *A. japonicus* belong to the Ralfsiales as a member of the family Ralfsiaceae.

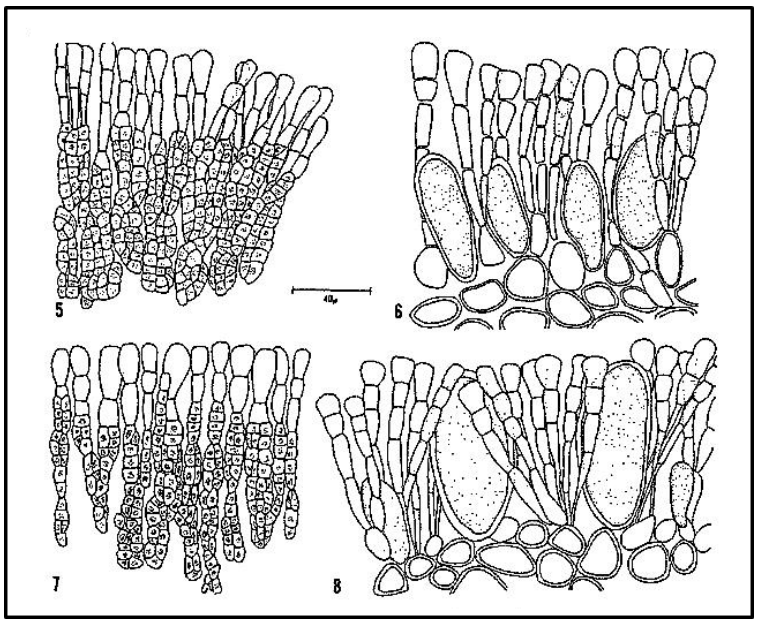

Figure 2.24 Row above: *Analipus filiformis* and row below: *A. japonicus*. Plurilocular structures are shown on the left while unilocular structures and paraphyses are shown on the right. (After Wynne 1971, p. 172, figs. 5- 8).

2.4.6.11 *Heteroralfsia* Kawai

Generitype: *Heteroralfsia saxicola* (Okamura & Yamada) Kawai (Fig. 2.25)

Type locality: Japan

Distribution: Endemic to temperate water of eastern Asia - Japan (Kawai, 1989, Lim et al., 2007); China (Zeng, 2009); Korea (Lee and Kang, 2001)

A monotypic genus. One of the two genera with an erect form of thalli, the other being *Analipus*. The taxonomic origins of *H. saxicola* can be traced back to *Gobia saxicola* Okamura & Yamada. Yamada (1928) placed this species in the genus *Gobia* based on its morphological similarity to *Gobia simplex* Setchell & Gardner. The species was then transferred to the genus *Saundersella* by Inagaki (1958) following the treatment of Kylin (1940) who transferred *G. simplex* to his new genus *Saundersella* which was

placed in the Chordariaceae, Chordariales. Kawai (1989) established the genus *Heteroralfsia* to accommodate the new combination *H. saxicola* (Okamura & Yamada) Kawai and placed it in the Ralfsiaceae but with some hesitation. The author noted that *Saundersella saxicola* (Okamura & Yamada) Inagaki showed discrepancies with *Saundersella simplex*, the generitype in having single sinuate cup-shaped or several fragmented chloroplast without pyrenoid per cell, mediate discal-type germination, intercalary plurilocular sporangia with a terminal sterile cell, cells with abundant physodes and almost isodiametric pigmented medullary filaments. In contrast, *Saundersella simplex* has a filamentous gametophyte forming terminal plurilocular sporangia, basal portion of erect thalli composed of rhizoidal filaments and cells containing several chloroplasts with obvious pyrenoids, characteristic of the Chordariales.

This heteromorphic genus is also characterised by its caespitose erect portions which are lost after maturation and basal crustose portions which also reproduces by forming plurilocular sporangia and thus maybe a potential (or reduced gametophytic) generation. The crustose portion is unilaterally constructed, composed of a basal layer and closely packed, erect filaments issuing from it and the surface of the crustose portion is covered with a thick cuticle. A transition is present between the relatively short cells of the crust and the elongate medullary cells of the erect portion of the thallus. Erect portions of thalli are cylindrical, roundish at the tip, slightly slimy, solid near the base and hollow in the middle or upper part. Unilocular sporangia are sessile on the basal cells of assimilatory filaments (paraphyses) which form from the cortical cells of the erect portions (regarded as a sporophytic generation). Plurilocular sporangia were not found on the erect portions of thalli. Independent gametophytic generations were not observed in the field or in culture. Swarmers from both unilocular and plurilocular sporangia undergo discal-type germination forming a crustose thallus. Mating reactions

between plurispores were not observed. Formation of uni- to biseriate, intercalary plurilocular sporangia with a terminal sterile cell, on the crustose portion takes place in winter. Kawai (1989) considers *H. saxicola* as an intermediate between *Ralfsia* (crustose thallus forming intercalary plurilocular sporangia) and *Analipus* (erect thallus forming unilocular sporangia). In a molecular study by Lim et al. (2007), *H. saxicola* was resolved as a member of Ralfsiales and was grouped in the clade corresponding to the family Ralfsiaceae along with *E. aurea*, *A. japonicus*, *R. fungiformis* and *R. verrucosa*.

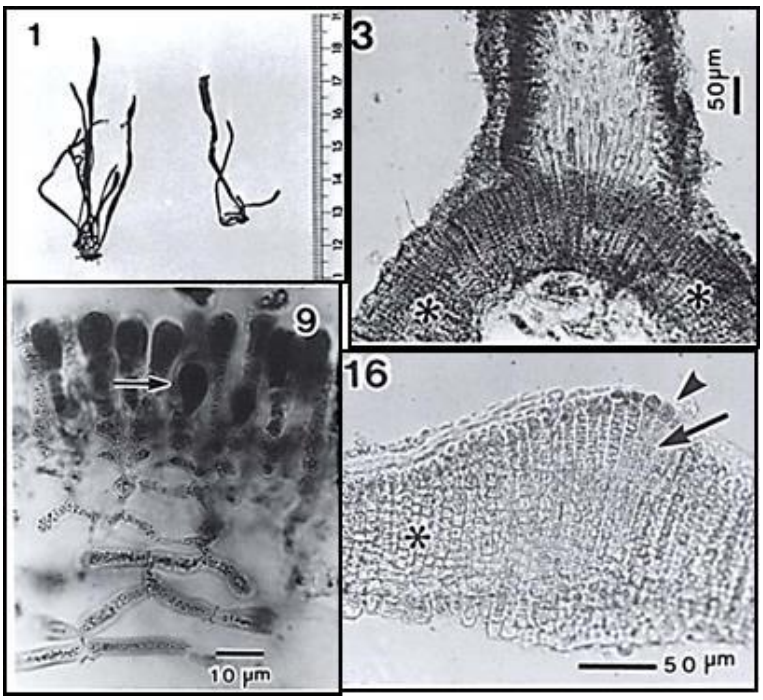

Figure 2.25 *Heteroralfsia saxicola*. Top left: Habit of the plant; Top right: Longitudinal section of the crustose holdfast (asterisks) and the erect thallus which issues from it; Bottom left: Cross section of mature erect thallus (arrow shows unilocular sporangium); Bottom right: Longitudinal section of mature crust (arrowhead shows cuticle and terminal sterile cells, arrow shows intercalary plurilocular sporangia, asterisk shows vegetative erect filament) [after Kawai 1989, p. 244 (figs. 1 and 3), p. 246 (fig. 9), p. 247 (fig. 16)]

2.4.6.12 *Jonssonia* Lund

Generitype: *Jonssonia pulvinata* Lund (Fig. 2.26)

Type locality: East Greenland

Distribution: East Greenland (Lund, 1959); Iceland (Caram and Jónsson, 1972).

A monotypic genus in which Lund (1959) was uncertain of its systematic position and provisionally placed it under the Ralfsiaceae. The generitype was described to be epiphytic on *Desmarestia aculeata* (Linnaeus) Lamouroux. According to Lund (1959), this alga is characterised by the "*firm structure of its pulvinate, parenchymatous thallus, the peripheral part of which is composed of few-celled, branched or simple, erect or slightly curved filaments bearing, usually terminally, free plurilocular or unilocular sporangia, solitary or arranged in short semi-intercalary rows*". Evacuation of the spores takes place apically for both types of sporangia. Lund (1959) described the plurilocular sporangia as the *Ectocarpus* type, more or less markedly articulated, uniseriate, subcylindrical or elongate conical, frequently curved and formed from few-celled shoots. Unilocular sporangia were subcylindrical or slightly clavate. The nature of the chloroplasts was undetermined.

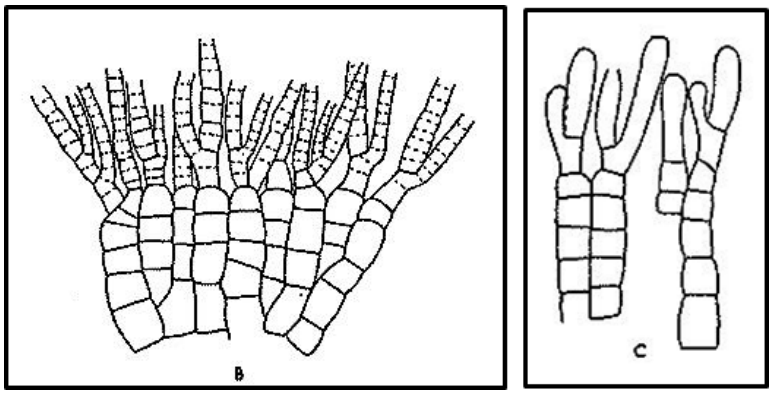

Figure 2.26 *Jonssonia pulvinata* shown with plurilocular sporangia (left) and unilocular sporangia (right) [after Lund 1959, p. 87, fig.15].

2.5 Confusion in the terminology of position of reproductive structures in crustose brown algae

An important taxonomic character in distinguishing species, genera and families of the crustose brown algae, is the position and organisation of reproductive structures. Nevertheless, confusion in the terminology used by various authors in their descriptions (for e.g., "terminal", "lateral", "stalk cells" and "paraphysis") often lead to varying interpretations when the terms are used for comparisons among taxa, even if the intended meaning may be clear for each taxon described *per se*. The proposed set of terms used to describe the position of reproductive structures by León-Alvarez and Norris (2005) for standardization in algal systematics is applied for this study and illustrated in Figs. 2.27 - 2.28.

León-Alvarez and Norris (2005) defined a "paraphysis" as a filament or a cell arising from a vegetative or reproductive filament adjacent to the reproductive structures, which may serve as protection, and can be morphologically differentiated from the supporting filament. The authors also consider "stalks" as cells or short filaments from which reproductive structures arise, and although they are generally morphologically differentiated from their generating filaments, this may not always be the case. These two ambiguous terms are usually applied with uncertainty and some workers (e.g., Abbott and Huisman, 2004) have referred to all erect filaments of *Hapalospongidion* as paraphyses. For the context of this study, structures are interpreted as "paraphyses" or "stalk cells" based on the definition used by León-Alvarez and Norris (2005) and the author's own judgement.

The terms "lateral" and "terminal" are terms commonly given different interpretations by various authors. For instance in Fig. 2.27 (g - h), the reproductive structure may be considered as lateral (in relation to the paraphysis) or terminal (in relation to the parent erect filament). Most authors applied the term "lateral" to describe

the position of a reproductive structure in relation to the parent filament or the paraphysis (e.g., Nakamura (1972) uses “lateral” to distinguish the unilocular reproductive structure of Ralfsiaceae from the other families; Fig. 2.27 b, c, g and h). The term “terminal” is often used to describe the position of reproductive structures in reference to the parent filament or surrounding filaments (e.g., Nakamura (1972) on the unilocular and plurilocular reproductive structures of Lithodermataceae; Fig. 2.27 d - f). Some authors use the term “lateral” without giving the point of reference (e.g., Setchell (1924) on the sporangia of *Mesospora pangoensis*).

The proposal by León-Alvarez and Norris (2005) involves distinguishing between a reproductive structure’s origin and its position. The term origin is restricted for use in relation to the point-of-origin of a reproductive structure on a stalk or parent filament (if it is sessile). Thus a reproductive structure can arise in one of three ways: **intercalary** (originating between two vegetative cells of the erect filament but not laterally; Fig. 2.27a), **lateral** (arising laterally on an intercalary cell of the erect filament; Fig. 2.27b) and **terminal** (Fig. 2.27d). The use of “position” is used to describe a reproductive structure’s position in relation to its parent filament (when stalks are present), paraphysis (when applicable) and surrounding filaments. If no stalks were present, the lateral origin on the erect filament is equivalent to the lateral position of the reproductive structure with respect to the erect filament (Fig. 2.27c), in this case the apical filament is not considered as a paraphysis. When surrounding filaments are present, the position of a reproductive structure with reference to the height of surrounding filaments can be categorised as basal (Fig. 2.28a - c), middle (Fig. 2.28d - f), sub-superficial (Fig. 2.28g - i) and superficial (Fig. 2.28j).

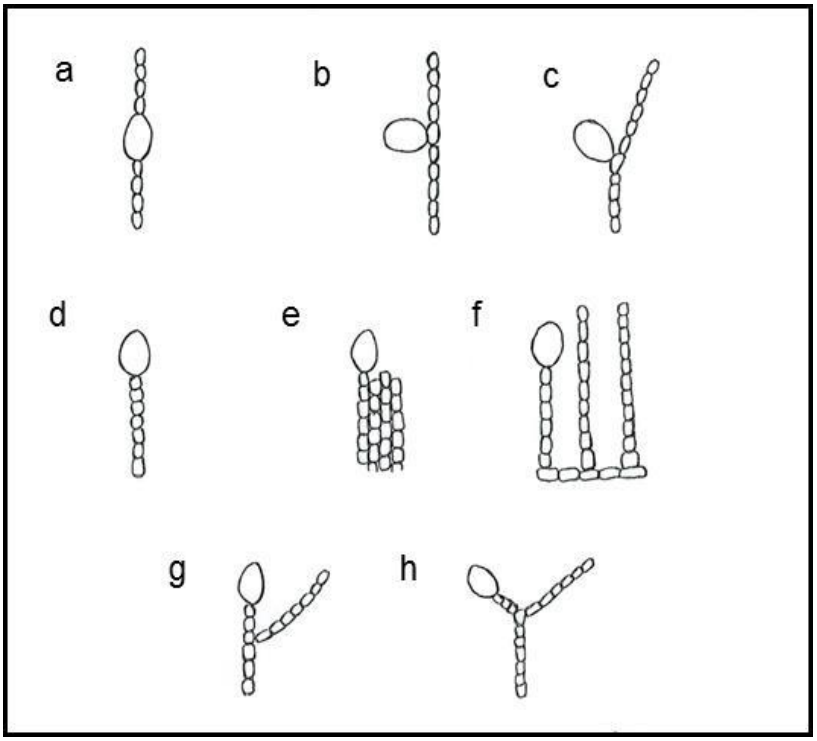

Figure 2.27 Origin and position of a reproductive structure on the stalk/parent filament and in relation to the parent filament/paraphysis (a) intercalary between the cells of the parent filament (b) laterally inserted on the parent filament (c) laterally inserted on the parent filament **or** terminally inserted on the parent filament and lateral-basal (sessile) to the paraphysis (d - e) terminally borne on the parent filament/stalk (f) terminally borne on parent filament/stalk cells arising directly from the basal plate (g - h) terminally inserted on stalk cells and lateral to the parent filament/paraphysis. (Adapted from León-Alvarez and Norris 2005, pp. 95 - 98, figs. 3 - 31 with modification)

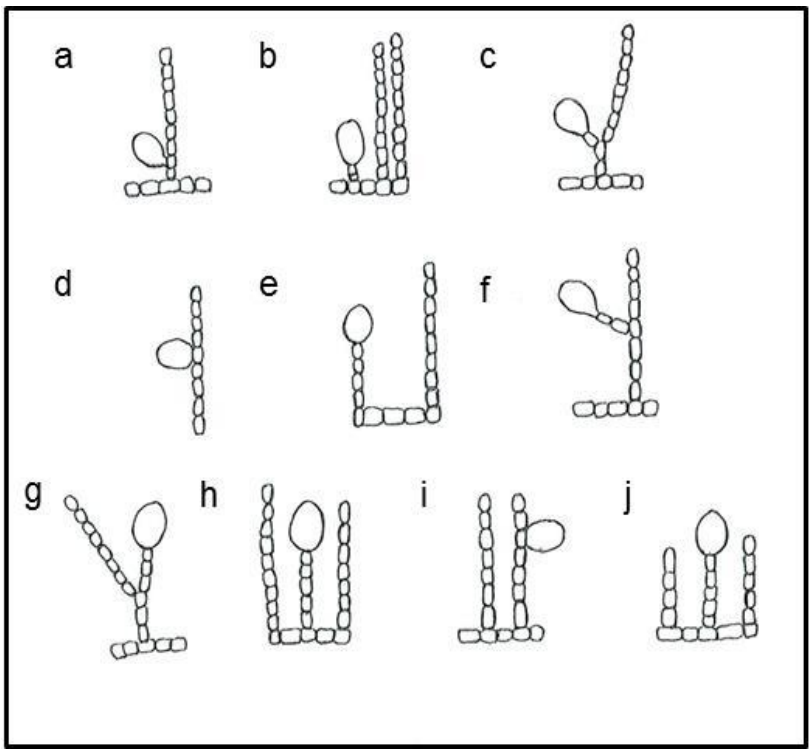

Figure 2.28 Position of a reproductive structure relative to the height of surrounding filaments (a - c) basal (d - f) middle (g - i) sub-superficial (j) superficial. (Adapted from León-Alvarez and Norris 2005, pp. 95 - 98, figs. 3 - 31 with modification)

2.6 Molecular studies involving the crustose brown algae

The Ralfsiales appears to be an order often overlooked by authors working on the molecular phylogeny of brown algae. Until the present, only a handful of studies included members of the Ralfsiales and even so, taxon sampling was limited to one or two members of this group (Tan and Druehl, 1994; Druehl et al., 1997; De Reviers and Rousseau, 1999; Cho et al., 2004; Cho et al., 2006; Bittner et al., 2008; Silberfeld et al., 2010) while a comprehensive study on the Ralfsiales was conducted by Lim et al. (2007). The first molecular study on members of Ralfsiales was conducted by Tan and Druehl (1994) using 18S rDNA. Their study showed that *Ralfsia fungiformis* and *Analipus japonicus* do not belong to the Ectocarpales but declined to offer an alternate

ordinal placement. Later, Cho et al. (2006) in their study using *ps*aA showed *A. japonicus* and *Nemoderma tingitanum* to be distantly related.

De Reviers et al. (2007) were the first to point out the non-monophyly of the Ralfsiaceae (Ralfsiales), Nemodermataceae (Nemodermatales) and Lithodermataceae (see Fig. 2.29). The genus *Petroderma* was placed with the Ishigeales near the root of the tree. In the same year, Lim and her co-workers (2007) presented the non-monophyly of the Ralfsiales and members of the order were split in two major groups, excluding Nemodermataceae and Lithodermataceae which were not included in the study (see Fig. 2.30). One group comprised members of the Ralfsiaceae, Mesosporaceae, *Analipus japonicus*, *Endoplura aurea* and *Heteroralfsia saxicola* whereas the other group contained species of *Diplura*, which were shown to be a sister group of the Ishigeales. They suggested the removal of *Diplura*, a genus originally placed in the Ralfsiaceae to a new family of its own in Ishigeales. On top of that, the authors established the family Neoralfsiaceae included in Ralfsiales to accommodate the new genus *Neoralfsia*, formerly known as *Ralfsia expansa* (J. Agardh) J. Agardh.

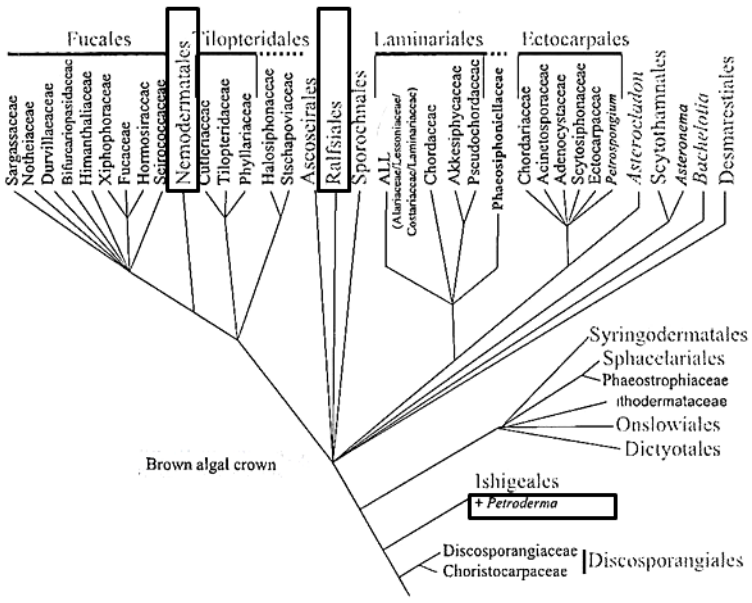

Figure 2.29 Synthetic tree presenting an update of brown algal phylogeny in 2006 (After De Reviers et al. 2007, p. 278, fig. 14.5).

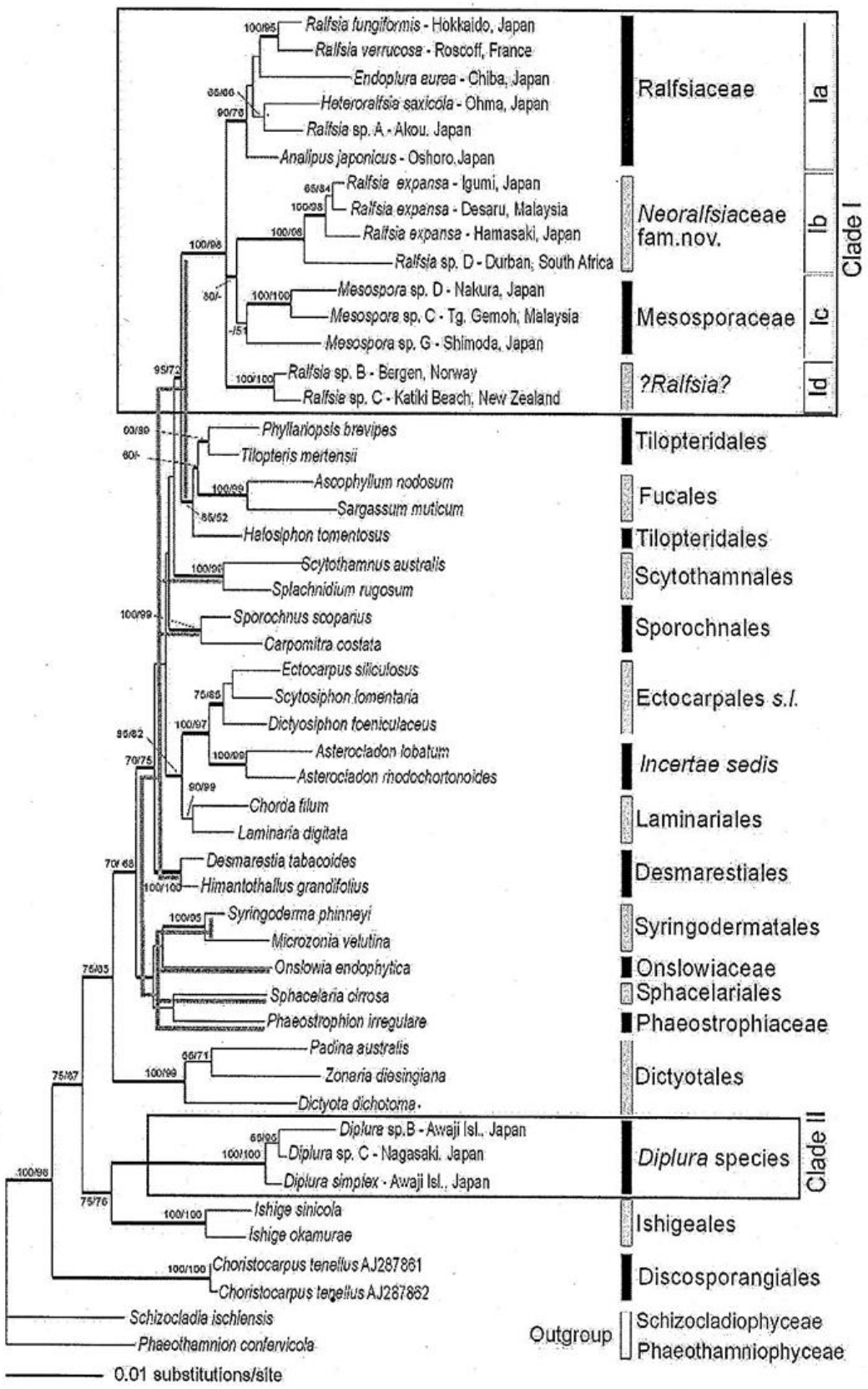

Figure 2.30 *rbc*L phylogenetic tree displaying the non-monophyly of the crustose brown algae (After Lim et al., 2007, p. 462, fig. 1).

Phillips et al. (2008) in their work using *rbc*L and 28S rDNA, proposed a new order Nemodermatales to accomodate *Nemoderma tingitanum*. This alga was previously classified in the monotypic family Nemodermataceae (Feldmann, 1937) which along with two other crustose brown algal families, the Ralfsiaceae and Lithodermataceae are included in Ralfsiales (Nakamura, 1972). The removal of *Nemoderma* from the Ralfsiaceae was justified by its number of chloroplasts (multiple plastids without pyrenoid), type of sexuality (isomorphic life cycle with marked anisogamy [Kuckuck, 1912]) and intercalary unilocular reproductive structures in contrast to Ralfsiaceae's single plate-like plastid (lacking pyrenoids), a direct life cycle and terminal unilocular reproductive structures. Like Cho et al. (2006), they demonstrated the distant relationship between *A. japonicus* and *N. tingitanum* but at the same time, *N. tingitanum* was found to form sister relationship with the Fucales whom they share multiple plastids lacking pyrenoids. A study by Silberfeld et al. (2010) on the phylogeny of brown algae supported the findings of Phillips et al. (2008), in which establishment of the order Nemodermatales is warranted (see Fig. 2.31).

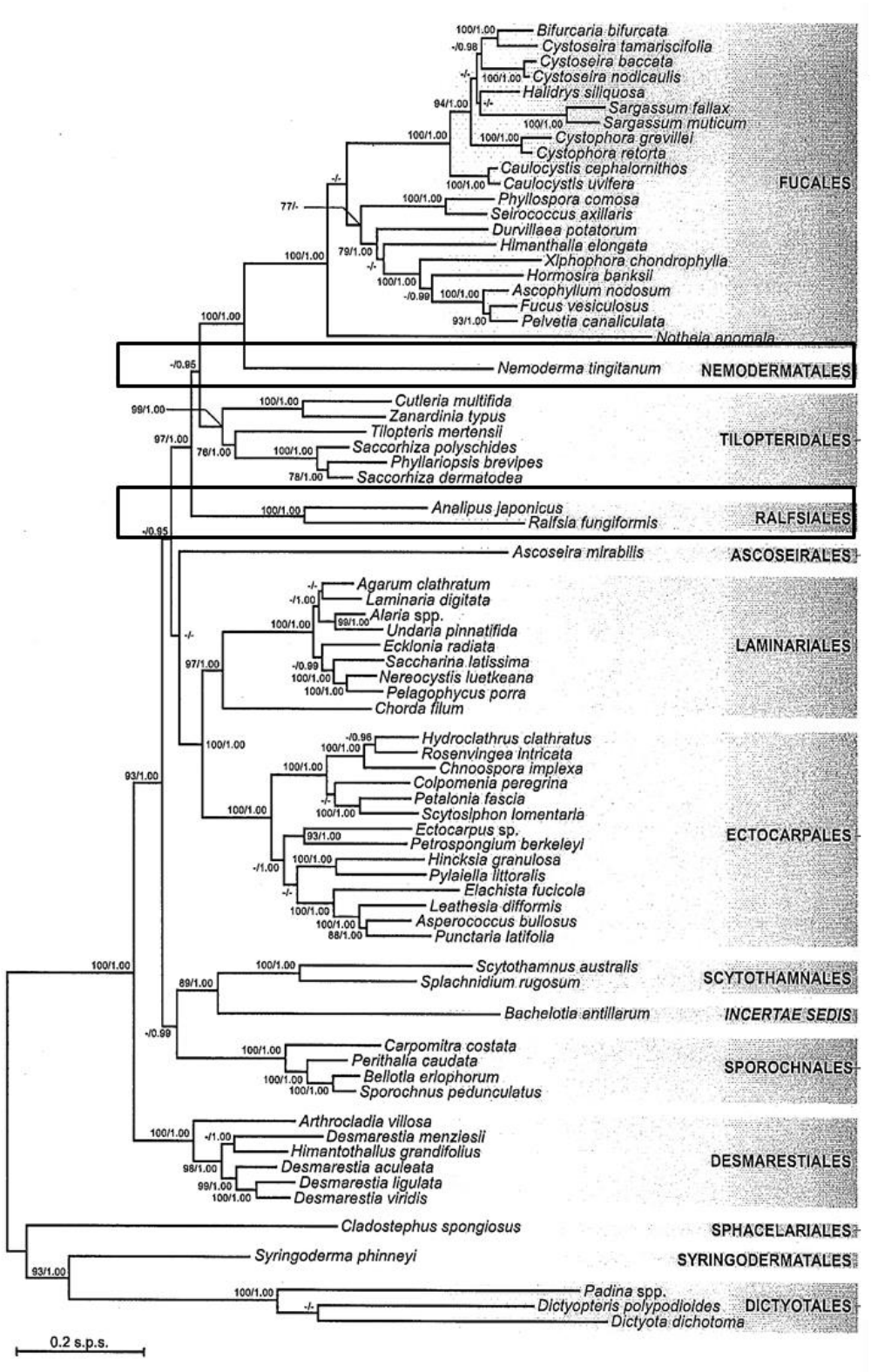

Figure 2.31 ML phylogram showing the separation of *Nemoderma tingitanum* from the Ralfsiales, which lead to establishment of the order Nemodermatales by Phillips et al., 2008 (After Silberfeld et al., 2010, p. 666, fig. 1).

In a study on the molecular phylogeny of the Dictyotales utilising markers from the nuclear, plastid and mitochondrial genome, Bittner et al. (2008) suggested the establishment of the order Petrodermatales to accommodate *Petroderma maculiforme* which was sister to the Ishigeales, however the relevant data is yet to be published (see Fig. 2.32). McCauley and Wehr (2007) conducted a study on freshwater brown algae which included two crust-forming brown algae: *Heribaudiella fluviatilis* and *Porterinema fluviatile* on the basis of *rbc*L sequences to ascertain their inclusion in the Ectocarpales. Their study indicated that the inclusion of *H. fluviatilis* in the Ectocarpales was not warranted since it formed a well-supported clade with the Syringodermatales and Sphacelariales and although the ordinal status of *P. fluviatile* was not resolved, it was largely distant from the Ectocarpales. Nonetheless, the diagnostic test for Sphacelariales (exposure to sodium hypochlorite) carried out on specimens of *Heribaudiella* tested negative.

The study by Bittner et al. (2008) also included *Heribaudiella fluviatilis* and *Pseudolithoderma roscoffense* which they labelled *incertae sedis* despite the taxa's placement in the Lithodermataceae at the time. The result of their study was consistent with that by McCauley and Wehr (2007) since both taxa were found to be sister taxa of the Sphacelariales. According to Parente et al. (2005; unpublished results presented at the VIIIth International Phycological Congress in Durban, South Africa), *Pseudolithoderma extensum* (Crouan & Crouan) Lund is not closely related to *P. roscoffense* and currently there is no DNA sequence data available for the type of the family i.e. *Lithoderma fatiscens* Areschoug.

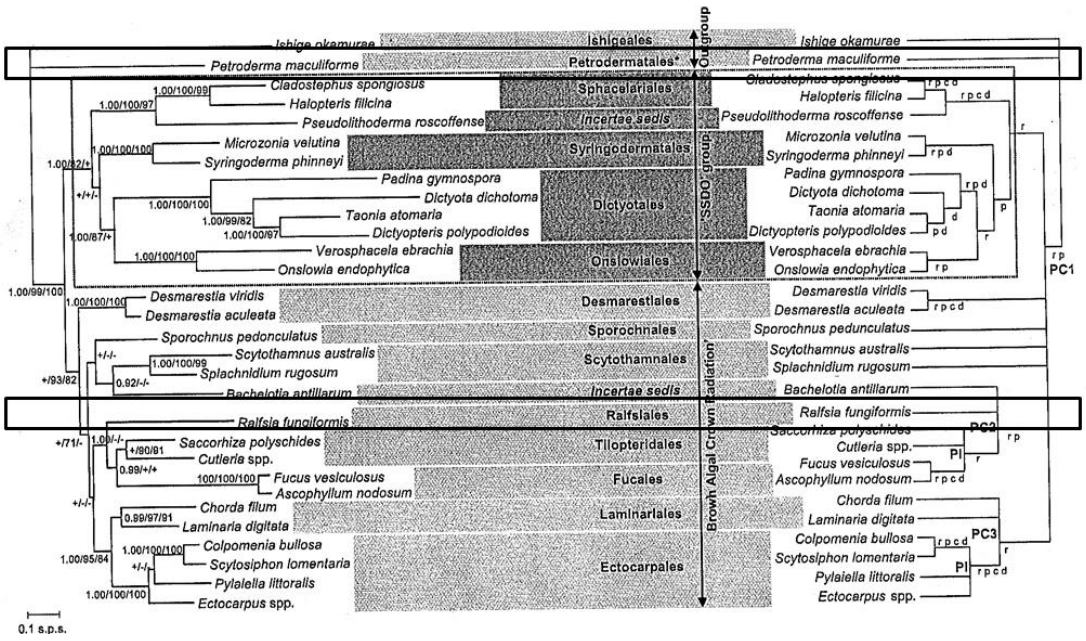

Figure 2.32 Bayesian phylogram and veto supertree obtained using partial LSU rDNA 5' end, *psa*A, *rbc*L and *cox*1-5' showing the exclusion of *P. maculiforme* from the Ralfsiales. Petrodermatales* are unpublished data. (After Bittner et al., 2008, p. 218, fig. 1).

2.7 Studies on crustose brown algae in the Indo-Pacific region

The crustose brown algae in Malaysia have received little attention despite detailed studies conducted on these algae since the 1800s with temperate species forming the bulk of the studies. Prior to the year 2007, there is no documentation of these algae in Malaysia. They were first reported in a study by Phang et al. (2007), in which only the genus *Ralfsia* was recorded and no details on the description and distribution of the species was available. Subsequently, species from two genera were mentioned in two studies involving crustose brown algae from Malaysia, namely *Neoralfsia expansa*, *Mesospora schmidtii* and *Mesospor*a sp. C (Lim et al., 2007; Lim et al., 2008). In the latter report, the authors' preliminary survey at various coastal areas in Malaysia found

the crustose brown algae to grow abundantly. Likewise, reports on crustose brown algae in Indonesia were limited to the studies by Weber-van Bosse (1911, 1913) whereby *M. schmidtii*, *N. expansa* (as *Ralfsia expansa* J. Agardh), two species of *Stragularia* [*S. clavata* (Harvey) Hamel and *S. polycarpa* Weber-van Bosse] and a putative species of *Lithoderma* were the only crustose brown algal taxa on record.

The Indo-Malay archipelago, located between the Indian and Pacific Ocean is well known as a marine biodiversity hotspot (Hoeksema, 2007). Yet, there are relatively few reports of crustose brown algal taxa from this enclave. The geographical coverage of the present study which include Peninsular Malaysia, Sabah (Borneo) and Lombok Island (Indonesia) are chosen for its location, the history of taxonomic work on crustose brown algal taxa and the manageable number of taxa. Lombok Island was also particularly sampled as it hosted two of the syntype localities of *M. schmidtii*. From the time of Weber-van Bosse's work until recently, only one new addition [i.e. *M. elongata* (Poong et al., 2013)] was made to the Indo-Malay crustose brown algal flora, clearly highlighting a need for this study.

From a wider biogeographic perspective, studies on crustose brown algae from the Indo-Pacific region (Fig. 2.33) are rather limited in comparison with their western counterparts. In fact, most reports concerning this group of algae from this region are from Japan (Tanaka and Chihara, 1980 - 1982).

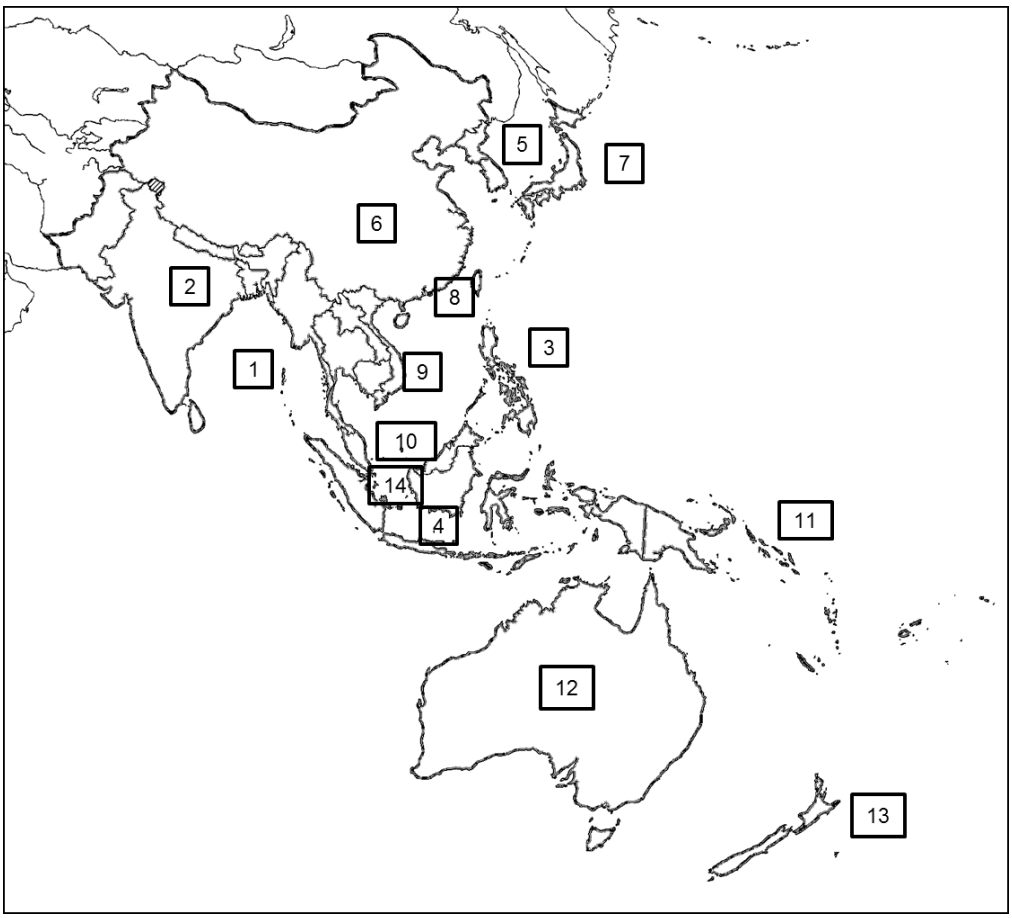

Figure 2.33 Studies on crustose brown algae in the Indo-Pacific region.

Legend:

Number on the map	Location	Taxa and references
1	Andaman Islands	*Mesospora schmidtii* (Krishnamurthy and Baluswami, 1986)
2	India	*Hapalospongidion thirumullavaramense, Pseudolithoderma thangasseriense* (S. Nettar and Panikar, 2009)
3	Philippines	*Ralfsia fungiformis, M. negrosensis* (Silva et al., 1987; West and Calumpong, 1996)
4	Indonesia	*M. schmidtii, M. elongata, Neoralfsia expansa, Stragularia clavata, S. polycarpa* (Weber-van Bosse, 1913; Poong et al., 2013, 2014)
5	South Korea	*A. japonicus, H. saxicola, N. expansa, Stragularia clavata, R. fungiformis, R. confusa, R. clavata, R. integra* (Lee and Kang, 2001; Keum, 2008)
6	China	*Heribaudiella fluviatilis, H. saxicola, R. huanghaiensis, R. verrucosa* (Hu and Wei, 2006; Zeng, 2009)
7	Japan	*Ralfsia fungiformis, R. verrucosa, R. bornetii, R. integra, R. endopluroides, R. pedicellata, N. expansa, Endoplura aurea, Diplura simplex, Pseudolithoderma subextensum*, *Analipus japonicus*, *Heteroralfsia saxicola*, *Ralfsia* spp., *Diplura* spp., *Mesospora* spp. (Tanaka and Chihara, 1980a- c; Tanaka and Chihara, 1981a- c; Tanaka and Chihara, 1982; Lim et al., 2007)
8	Hong Kong SAR	*M. schmidtii, D. simplex, E. aurea, P. subextensum, N. expansa, H. gelatinosum, R. endopluroides, R. tenuis, R. verrucosa* (Kaehler, 1994; 1998)
9	Vietnam	*M. schmidtii, N. expansa, R. fungiformis, R. verrucosa* (Dawson, 1954;Van Nguyen et al., 2013)
10	Malaysia	*M. schmidtii*, *M. negrosensis, Mesospora* sp. C, *N. expansa, Diplura* spp. (Lim et al., 2007; Poong et al., 2013, 2014)
11	Solomon Islands	*M. schmidtii* (Womersley and Bailey, 1970)
12	Australia	*R. verrucosa, P. australe, H. capitatum, M. schmidtii, N. expansa* (Womersley, 1987; Kraft, 2009; Bostock and Holland, 2010)
13	New Zealand	*H. saxigenum, P. roscoffense, R. verrucosa, R. confusa, Ralfsia* sp., *N. expansa*, *Diplura* sp. (Lindauer, 1949; Lindauer, 1961; Buchanan, 2005)
14	Singapore	*N. expansa* (Pham et al., 2011)

CHAPTER 3: METHODOLOGY

3.1 Field sampling and processing

Collections of crustose brown algae were made from May 2009 to July 2012. Figure 3.1 illustrated the sampling sites in this study. Brown algal crusts (mostly on rocks) were randomly collected along the west and east coasts and several islands off Peninsular Malaysia (Fig. 3.2): Pulau Sayak, Kedah; KM 16, Port Dickson, Negri Sembilan; Pantai Dickson, Port Dickson, Negri Sembilan; Telok Pelandok, Port Dickson, Negri Sembilan; Pulau Besar, off Malacca; Pantai Chendering, Terengganu; Pantai Kemasik, Terengganu; Telok Kalong, Terengganu; Teluk Sari, Johor; Teluk Ramunia, Johor (Fig. 3.3); Pulau Che Kamat, off Johor (Fig. 3.4); Pelabuhan Tanjung Langsat, Johor and Pulau Merambong, off Johor. Field collection was also conducted at the eastern part of Sabah on the coast of Sandakan and on several islands off the coasts of Semporna and Kunak (Fig. 3.5): Kampong Dandulit, Sandakan; Site 11, Semporna; Site 13, Kunak; Site 21, Semporna; Site 24, Pulau Bohey Dulang, Semporna (Fig. 3.6); Site 25, Pulau Silungun, Semporna (Fig. 3.7). A substantial collection was also made from various localities along the coasts of Lombok Island from the West Nusa Tenggara province of Indonesia (Fig. 3.8): Nipah, Lendang Luar, Batu Layar, Gili Genting, Batukijok, Labuhan Pandan and Rambang (Fig. 3.9) with the main intention of recollecting the type species of *Mesospora* from its syntype localities. Several specimens collected by Dr P.E. Lim from Japan (Fig. 3.10) and North America were also included in this study for comparison purposes.

Field-collected algal thalli attached to their substrata were air-dried prior to desiccation in silica gel and maintained at ambient temperature. Thalli attached to rocks too large to be transported back to the laboratory were collected by breaking off pieces

of the rock and if this failed, parts of the thalli were scraped off the rock with razor blades and kept in microcentrifuge tubes. Voucher specimens labelled "PSM" listed in Appendix A were deposited in the University of Malaya Seaweeds and Seagrasses Herbarium (KLU). The culture strain of *Mesospora negrosensis* (labelled "KU") and two voucher specimens (labelled "KU-d") currently identified as *M. elongata,* from the Kobe University Macroalgal Culture Collection (KU-MACC) and herbarium of the Kobe University Research Center for Inland Seas (KURCIS) respectively, were provided by Dr Hiroshi Kawai (Kobe) and used for DNA extraction. A specimen of *Hapalospongidion saxigenum* from its type locality of Stewart Island, New Zealand was provided by Dr Wendy Nelson (Wellington) and was used for DNA extraction. Dr Stefano G.A. Draisma provided two specimens from a neighbouring island west of Alor Island, Indonesia.

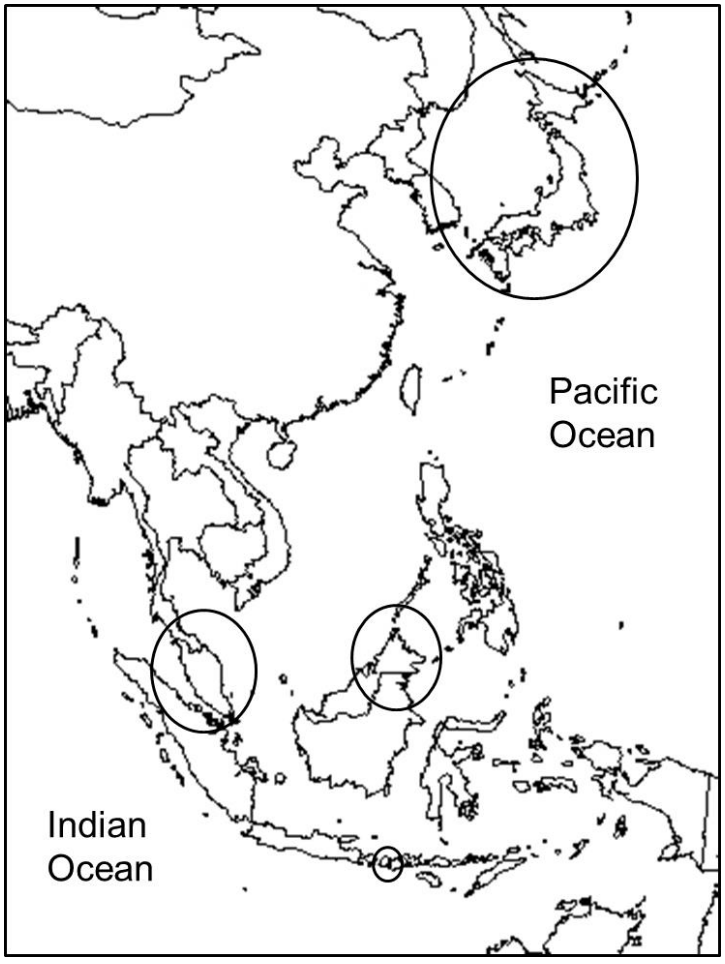

Figure 3.1 Map showing the collection sites (circled) of specimens in this study.

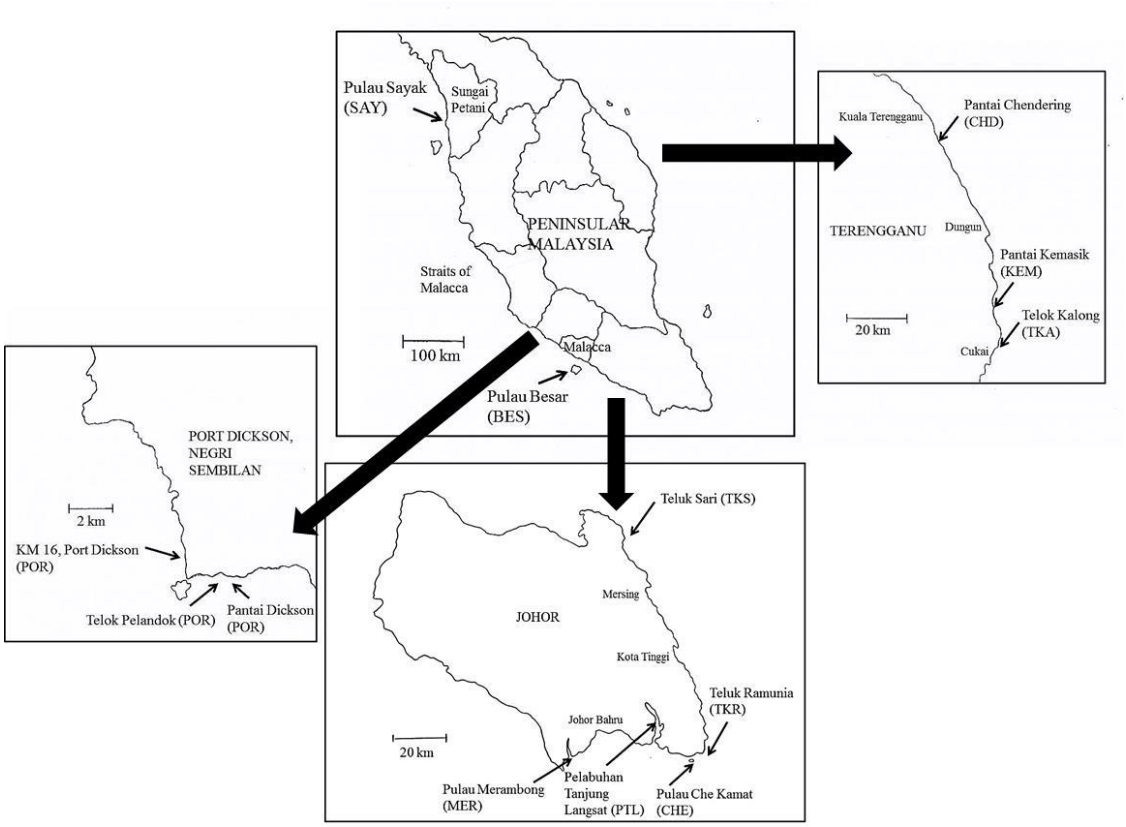

Figure 3.2 Map of collection sites in Peninsular Malaysia.

Figure 3.3 Collection site at Teluk Ramunia, Johor. Photograph courtesy of P.K. Ng.

Figure 3.4 Collection site at Pulau Che Kamat, Johor. Photograph courtesy of P.K. Ng.

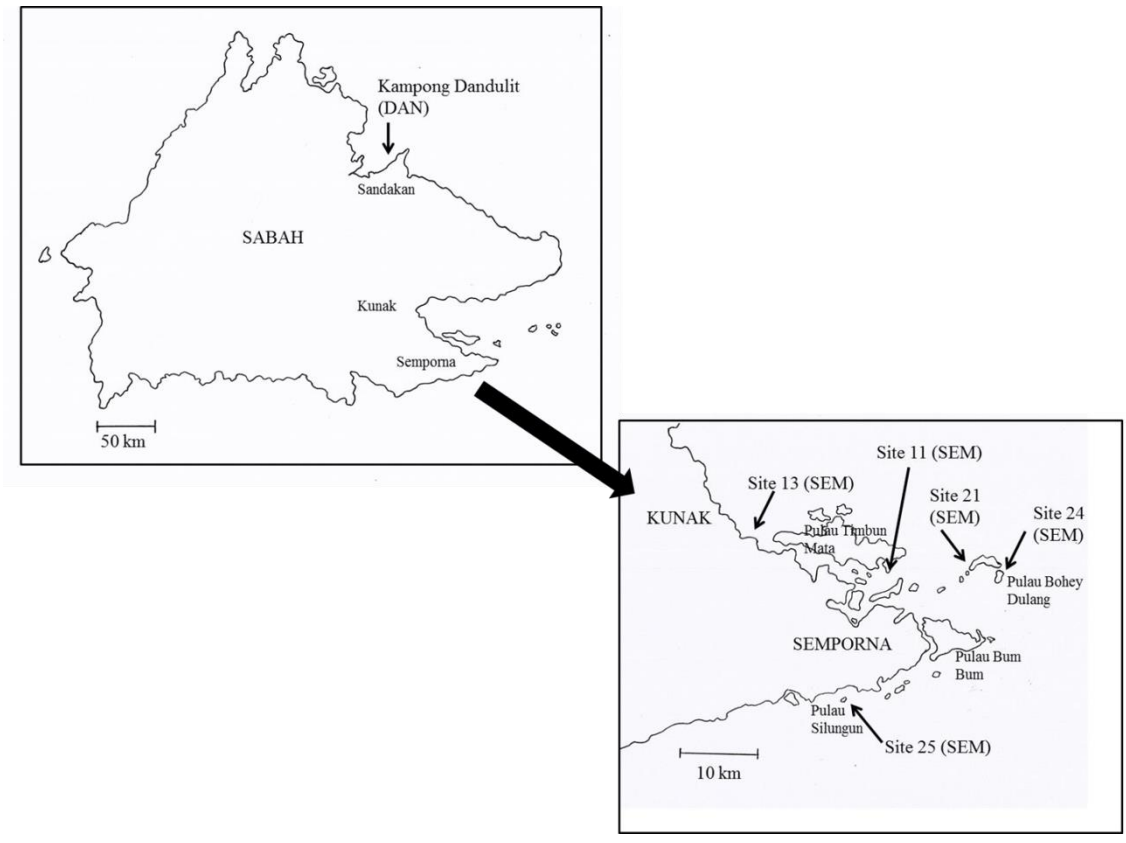

Figure 3.5 Map of collection sites in east Sabah.

Figure 3.6 Collection site at Pulau Bohey Dulang (Site 24), Semporna, Sabah. Photographs courtesy of P.K. Ng.

Figure 3.7 Collection site at Pulau Silungun (Site 25), Semporna, Sabah. Photograph courtesy of P.K. Ng.

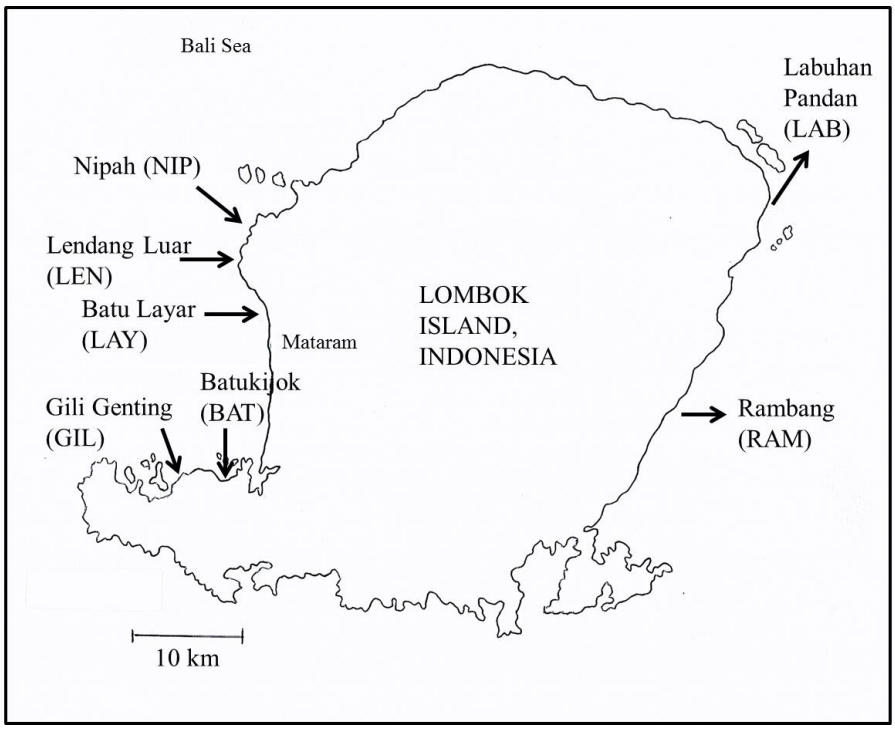

Figure 3.8 Map of collection sites on Lombok Island, Indonesia.

Figure 3.9 Collection site at Rambang, Lombok Island, Indonesia.

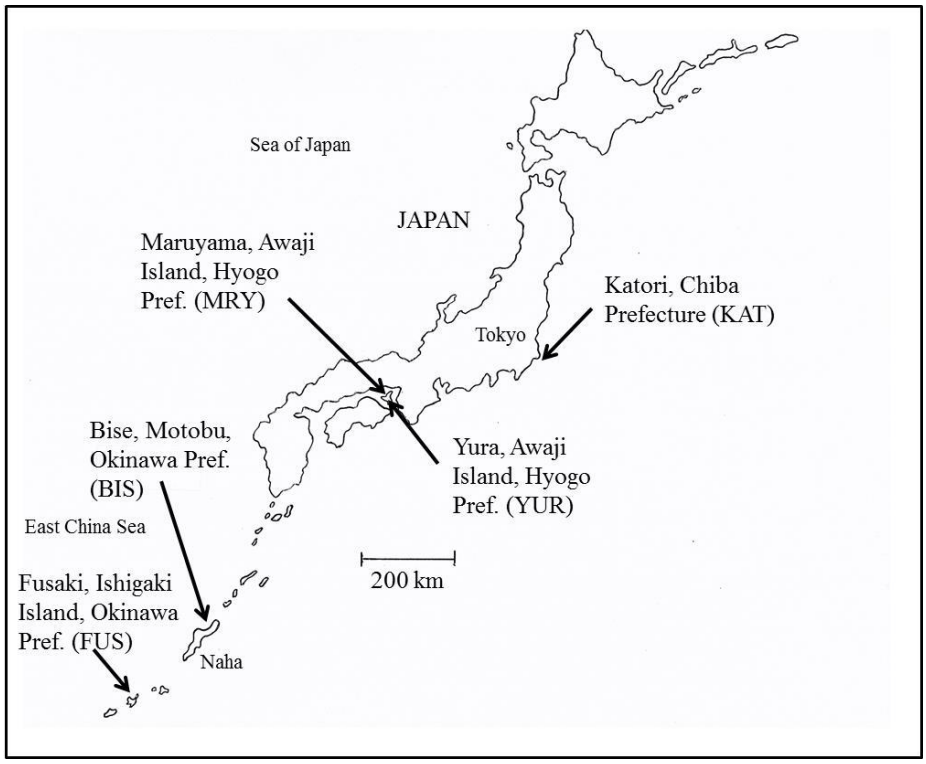

Figure 3.10 Map of collection sites in Japan.

3.2 Morpho-anatomical observations

The thalli were first moistened with sea water to identify possible fertile spots which can significantly aid identification. Intact algae were then removed from the substratum (rocks or shells) with a razor blade. Careful attention was needed to ensure isolation of a single species from the confluent thalli of two or more different species, which were common in this study. Squash preparations mounted in seawater were observed under the light microscope. Characters examined for species identification were relatively few due to the simple anatomy of the specimens and these included number and size of cells of the erect filaments, position and dimensions of unilocular and plurilocular reproductive structures, number of sterile terminal cell(s) and number of stalk cell(s). Photomicrographs were taken using a DP72 digital camera attached to a BX51 microscope (Olympus, Japan) while measurements were made using the measureIT software (Olympus Soft Imaging Solutions, Germany). Squash preparations mounted in

corn syrup as permanent slides were deposited in the University of Malaya Seaweeds and Seagrasses Herbarium (KLU). A small portion of thallus was extracted from the same spot that was examined for morpho-anatomical observations and used for DNA isolation.

3.3 Molecular genetic analyses

3.3.1 Genomic DNA isolation

Approximately 3 - 5 mg of dried thalli were ground in liquid nitrogen and genomic DNA was extracted using the *i*-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology Inc., South Korea) following the manufacturer's Type B protocol (for fresh, dried or frozen leaf) but with slight modifications. Prior to incubation at 65°C, the ground thalli added with lysis buffer is subjected to sonication at high frequency for 10-15 mins to enhance cell lysis and release of DNA. The incubation time at 65°C was prolonged to between 45 - 60 mins whereas the incubation in ice was prolonged to 15 mins. At the DNA binding step, instead of the recommended 200 µl of lysate to be recovered for the following step, maximum recovery of the lysate (400~450 µl) without touching the cell-debris pellet was performed to obtain a higher yield of DNA. The precipitation and washing steps were done according to the manufacturer's protocol. For the elution step, DNA was eluted in two fractions of 100 µl and the second fraction was used in PCR reactions.

3.3.2 Determination of DNA yield and quality using UV spectrophotometry

Spectrophotometric measurement of UV absorption at wavelengths 230, 260 and 280 nm using a BioPhotometer (Eppendorf, Germany) was carried out to estimate the yield and quality of isolated DNA. An optical density (OD) of 1 at A_{260} corresponds to a concentration of 50 µg/ ml for double stranded DNA. The ratio of the absorbance at 260 nm and 280 nm ($A_{260/280}$) provide an indication of protein contamination while $A_{260/230}$ is used to indicate polyphenol and carbohydrate contamination. The $A_{260/280}$ value for a pure DNA solution is approximately 1.8 ± 1 (Vinod, 2004).

3.3.3 Polymerase chain reaction (PCR) amplification

DNA amplification of the *rbc*L gene (~1350 nucleotides) and partial *cox*1-5' gene (~ 650 nucleotides) was carried out in an Eppendorf Mastercycler Gradient EP S thermal cycler (Eppendorf, Germany) using the *i*-taq plus PCR kit (iNtRON Biotechnology Inc., South Korea). Total volume of the PCR reaction mix was 20 µl containing 1 - 2 µl (25–50 ng) diluted or non-diluted template, 2 µl of 10x *i*-taq plus reaction buffer, 0.75 µl of dNTP mixture (consisting 2.5 mM each of the dNTP), 1 µl of each forward and reverse primers (10 pmol/ µl), 0.25 µl of *i*-taq plus DNA polymerase and adjusted to 20 µl with ultrapure water. The optimum DNA concentration used for PCR ranged from ten times dilution with ultrapure water to 2 µl of the extracted DNA, depending on the amount of raw material used during DNA extraction.

DNA extracts of *Mesospora* sp. C, *Diplura* sp. B, *D. simplex*, *Neoralfsia expansa* (Desaru, Malaysia and Hamasaki, Japan) from Lim et al. (2007) were available and used for amplification and sequencing of *cox*1-5' gene. The original specimens and

DNA extracts of *Mesospora* sp. D, *Mesospora* sp. G and *Diplura* sp. C were not available for this study and therefore their *cox*1-5' sequences could not be determined.

Genetic markers from the nuclear genome such as SSU and LSU rDNA were not used in this study as previous works (e.g.: Rousseau et al., 2000; Draisma et al., 2001) using these markers indicated a sub-optimal resolution at the family and order levels. Meanwhile, the use of more variable nuclear markers such as ITS regions was not attempted due to difficulties in sequence alignment between distantly related taxa, as was expected for specimens of this study.

3.3.3.1 Amplification parameters for *rbc*L gene

The *rbc*L gene was amplified as two or three overlapping fragments using the primers listed in Table 3.1. New primers were designed using the Primer3 software (Untergrasser et al., 2012). The primer rbcR2 was used as an internal sequencing primer for PCR products amplified using the primer pair NDrbcL2/NDrbcL9. Some taxa required species-specific primer pair for successful amplification. They include: RspBF2/RspBR2 for amplification of the middle portion of the gene in *Ralfsia* sp. 1; DP_F7/DP_R544 for amplification of the 5' end in *Diplur*a sp. 2 and Sam4F1/SAM4R1 for amplification of the 5' end in the unidentified crustose brown alga species 1.

For the amplification with NDrbcL2/NDrbcL9, an initial denaturation for 3 mins at 94°C was followed by 5 cycles of denaturation at 94°C for 30 secs, annealing at 47°C for 30 secs, extension at 72°C for 1 min; followed by 35 cycles of denaturation at 94°C for 30 secs, annealing at 50°C for 30 secs, extension at 72°C for 1 min; and a final extension at 72°C for 10 mins. The profile for thermal cycling performed using other primer pair combinations is as follows: initial denaturation for 3 mins at 94°C followed

by 40 cycles of denaturation at 94°C for 45 secs, annealing at 50°C for 30 secs, extension at 72°C for 1 min; and a final extension at 72°C for 7 mins.

Table 3.1 Primers for amplification and sequencing of *rbc*L gene.

Primer name	Primer Sequence (5'-3')	Annealing position	Reference
Forward primers			
rbcF0	ATCGAACTCGAATAAAAAGTGA	20-41	Kawai and Sasaki, 2004
rbcF1	CGTTACGAATCWGGTG	43-58	Kawai and Sasaki, 2004
PRBF2	TTCCAAGGYCCHGCAACYGGT	454-474	Kogame et al., 1999
rbcF2	AGGTTCWCTWGCTAA	342-356	Kawai and Sasaki, 2004
RspBF2	TACGGTCGTGTTGTTTATGA	565-584	This study
RalF2	GCAACAGGTGTTATTGTAGA	466-485	This study
NDrbcL2	AAAAGTGACCGTTATGAATC	34-53	Daugbjerg and Andersen, 1997
PRBF3	TGTAAATGGATGCGTATGTG	952-971	Kogame et al., 1999
rbcF4	GTAAATGGATGCGTA	953-967	Kawai and Sasaki, 2004
DipF1	CAGGAGTAATTCCTTATGC	53-71	This study
DP_F7	CATACGCTAAAATGGGCTAT	65-85	This study
SAM4_F1	TCAGGTGTAATTCCTTACG	52-70	This study
DipB_F3	TMCATCGHGCAGGAAATTCT	887-906	This study
Reverse primers			
RalR952	CATACGACTCCATTTACA	952-934	Lim et al., 2007
rbcR2	CGCATGAATGGTTGTG	650-653	Kawai and Sasaki, 2004
PRBR2	CCTTTAAACCATTAAGGGATC	1040-1021	Kogame et al., 1999
NDrbcL9	GTACCACCACCAAAT	1226-1212	Daugbjerg and Andersen, 1997
PRBR3	GTAATATCTTTCCATAAATCTAA	1406-1384	Kogame et al., 1999

Table 3.1 (continued)

Primer name	Primer Sequence (5'-3')	Annealing position	Reference
RspBR2	AGTCGCACCTGATTGAATAC	1263-1244	This study
RalR2	CATTTGACCACAGTGAATAC	1171-1152	This study
RSPR	AATAAGGAAGACCCCATAATTCCCA	167-142[1]	Kogame et al., 1999
DipR1	GTTTACACCCTCCATACAAT	691-672	This study
DP_R544	AGTCCACCTCTTAAGCCTTC	602-583	This study
SAM4_R1	GTACGAACCTTTAACTTCAC	730-711	This study
DipB_R3	ACGAARTCTGGAGTATCTGTTG	1442-1421	This study

[1] Annealing position in *rbc*S

3.3.3.2 Amplification parameters for *cox*1-5' gene

Approximately 650 nucleotides of the 5' end of the *cox*1 gene was amplified as a single fragment using the primers listed in Table 3.2. Amplification for *Ralfsia* sp. 1 and "*Mesospor*a" sp. 3 can only be accomplished using the primer pair L/GazR2. The PCR conditions consisted of an initial denaturation for 3 mins at 94°C followed by 40 cycles of denaturation at 94°C for 45 secs, annealing for 30 secs at the temperatures presented in Table 3.2, extension at 72°C for 1 min; and a final extension at 72°C for 7 mins.

Table 3.2 Primers for amplification and sequencing of *cox*1-5' gene

Primer name	Primer Sequence (5'-3')	Direction	Annealing temperature	Reference
117F	TTTCHACNAAYCAYAAAGATAT	F	49°C	Bittner et al., 2008
GazF2	CCAACCAYAAAGATATWGGTAC	F	50°C	Lane et al., 2007
L	GGTCAACAAATCATAAAGATATTGG	F	50°C	Folmer et al., 1994
784R	TNTAYCARCATTTATTTTGGTT	R	49°C	Bittner et al., 2008
GazR2	GGATGACCAAARAACCAAAA	R	50°C	Lane et al., 2007
H	TAAACTTCAGGGTGACCAAAAAATCA	R	50°C	Folmer et al., 1994

3.3.4 Determination of yield and quality of the amplified product by agarose gel electrophoresis, purification of the amplified product and gene sequencing

PCR products were checked for length and yield by electrophoresis on 1% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, USA). The resulting products were directly purified using the LaboPass Gel and PCR Clean-up kit (Cosmo Genetech, South Korea). Alternatively, desired bands were excised from the agarose gel and DNA was extracted using the same purification kit. Cycle sequencing reactions were performed using the same primers used for PCR amplification and the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA). Forward and reverse sequences were determined by capillary sequencing using an Applied Biosystems 3730xl DNA Analyzer, conducted by 1st BASE Laboratories Sdn. Bhd. (Malaysia).

3.3.5 Analysis of sequence data

The raw sequences were assembled and edited in the program ChromasPro ver. 1.42 (Technelysium Pty Ltd, Australia). If a nucleotide could not be unambiguously determined from the chromatograms, the site was coded with IUPAC ambiguity codes and was treated as uncertainty in the analyses. Consensus sequences were preliminarily aligned in ClustalX v. 2.0.8 (Larkin et al., 2007) and subsequently manually revised in Bioedit v. 7.0.9.0 (Hall, 1999). The 5' and 3' ends were pruned from the alignment, because they contained a number of undetermined nucleotides for several taxa. ML, MP and BI analyses were performed for each gene separately, and for the two genes combined. List of representatives from each brown algal orders used in the phylogenetic analyses is given in Appendix B.

Analyses of *rbc*L alignment alone included sequences of 115 taxa of which 62 sequences were generated from this study and 53 were previously published sequences. The *cox*1-5' alignment alone comprised 75 crustose brown algal taxa of which 71 were sequences generated from this study and four were previously published sequences. Other brown algal taxa have been excluded from the *cox*1 analyses because these taxa cannot be placed reliably in the trees due to the accelerated evolutionary rate of the *cox*1 gene. The *rbc*L analyses used similar outgroups as the combined data set whereas the *cox*1-5' analyses employed *Ishige okamurae* Yendo as the outgroup. The resulting phylogenies were screened for significant topological incongruency (conflicting relationships with supported nodes) to assess the feasibility of combining sequences from the two genes. As the single gene trees did not show any supported conflicting nodes, the combined data set was used to generate phylogenetic trees. Combined analyses included 78 brown algal taxa plus three outgroup taxa (*Schizocladia ischiensis* Henry, Okuda & Kawai; *Phaeothamnion confervicola* Lagerheim and *Tribonema aequale* Pascher).

MP analyses were accomplished in PAUP 4.0b10 (Swofford, 2002) using heuristic search with 100 random sequence addition replicates and a tree bisection reconnection (TBR) branch-swapping algorithm. Gaps in the alignment were treated as missing data. All characters were treated as unordered and equally weighted, the Multrees option active and branches with a maximum length of zero collapsed to yield polytomies. To assess support for the resulting nodes, bootstrap percentage (BP) was computed with 1,000 replications using one random taxon additions under the heuristic search method with TBR swapping.

Kakusan v.3 (Tanabe, 2007) was used to determine the best-fit nucleotide substitution models in each codon position of each gene for each data set by comparing different evolutionary models via the corrected Akaike information criterion (AICc)

(Akaike, 1974) for ML analysis and the Bayesian Information Criterion (BIC) (Schwarz, 1978) for the BI analysis. The models and parameters selected are shown in Table 3.3. ML trees were inferred using Treefinder v. October 2008 (Jobb et al., 2004) with bootstrap percentage generated from 1,000 resamplings to estimate robustness.

BI analyses were conducted using MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) and the program was set to run four chains of Markov chain Monte Carlo iterations for 2,000,000 generations. Markov chains were sampled every 100^{th} generation. The average standard deviation of split frequencies decreased below 0.01 (indicating that the two runs had reach convergence) within 100,000 generations. The plot of generation versus log probability was inspected after the run using Tracer v.1.5 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure that stationarity was reached and to determine a suitable burn-in value. The first 2,000 trees sampled were discarded as "burn-in" to ensure stabilisation. The majority-rule consensus tree containing posterior probabilities was built from the remaining sampled trees.

The bootstrap approach (Felsenstein, 1985) which involves a random resampling of characters from the data set with replacement is applied in this study. Each new data set is reanalysed resulting in a new phylogeny and this process of resampling and analysis is then repeated for 1,000 times. A 50% majority-rule consensus tree is produced from all resulting trees and the bootstrap support expressed as percentage. For the purpose of comparison with bootstrapping, nodes with Bayesian posterior probabilities, PP >0.95 (the node appears in greater than 95% of sampled trees) are implied as being strongly supported, 0.90 - 0.95 as moderately supported and <0.90 as weakly supported. ML BP and MP BP are described as strong (≥ 85%), moderate (70% - 85%) and weak (< 70%). To assess levels of intra- and interspecific variations in the *rbc*L and *cox*1-5' sequences, uncorrected (*p*) pairwise genetic distances were estimated using PAUP 4.0b10 (Swofford, 2002).

Table 3.3 Model and parameters selected by Kakusan3 for ML analysis of combined *rbc*L-*cox*1-5', *rbc*L only and *cox*1-5'only data sets.

Data set	Model selected	-ln likelihood	Nucleotide frequencies				Substitution model rate matrix						Gamma distribution shape parameter (alpha)
			A	C	G	T	TC	TA	TG	CA	CG	AG	
combined *rbc*L-*cox*1-5'	GTR + G	42888.62	0.25	0.25	0.25	0.25	0.3692	0.2036	0.0587	0.0480	0.0537	0.2669	0.3004
*rbc*L only	GTR + G	26205.8	0.3567	0.0900	0.1042	0.4990	0.4622	0.0285	0.0488	0.0599	0.1418	0.2588	0.1965
*cox*1-5'only	J1 + G	10646.29	0.2660	0.1526	0.1570	0.4244	0.5826	0.0278	0.0278	0.0655	0.0655	0.2309	0.1642

CHAPTER 4: RESULTS

4.1 Determination of yield and quality of isolated DNA

Concentration of DNA isolated is in the range of 10 – 34 ng/µl while DNA purity as measured by A_{260}/A_{280} readings is in the range of 1.40 – 1.98.

4.2 Molecular phylogenetic analyses

4.2.1 Analysis of *rbc*L gene sequence data set

The length of the *rbc*L alignment was 1,349 nucleotides of which 709 (53%) were variable sites and 560 (41.5%) were parsimony-informative sites. MP analysis resulted in 17 equally most parsimonious trees and tree length was 5,883, consistency index (CI) was 0.2094 and retention index (RI) was 0.6696. The intraspecific genetic variation (Table 4.1) ranged from 0 - 0.15% in *Mesospora* sp. 2 to 0 - 3.89% in *M. schmidtii* (Appendix C). There were no intraspecific divergences within samples of *Ralfsia* sp. 1. Interspecific divergences within the genus *Diplura* ranged from 7.90 - 8.20% between *Diplura* sp. 1 and *Diplura* sp. 2 to 17.82 - 18.76% between *Diplura* sp. B and *Diplura* sp.4. The interspecific variation between the Japanese *Diplura* specimens were rather small numbering at 2.99% between *Diplura* sp. C and *D. simplex* to 5.27% between *Diplura* sp. B and *Diplura* sp. C. Within *Mesospora*, interspecific divergences ranged from 4.32 – 6.92% between *Mesospora* sp. 1 and *M. negrosensis* to 8.76 – 9.32% between *M. elongata* and *Mesospora* sp. D. The genetic variation between the putative new genus ("*Mesospora"* sp. 3) and the seven species of *Mesospora* ranged from 9.50% to 11.74%. *Mesospora* sp. G and "*Mesospora"* sp. 3 are closely related as shown by

their genetic variation of 8.69 - 9.02%. Pairwise distance between species of *Ralfsia* was 0.07% between *Ralfsia* sp. B and *Ralfsia* sp. 1 to 10.46% between *Ralfsia* sp. C and *R. fungiformis*. The *rbc*L gene was successfully sequenced for 166 specimens (100% success). There was essentially no alignment problem except for the insertion of two gaps at positions 548 and 636 to accommodate *Nemoderma tingitanum* (EF990253) and *Microzonia velutina* (Harvey) J. Agardh (AY157697), respectively.

Table 4.1 Intra- and interspecific divergences of *rbc*L and *cox*1-5' sequences of the specimens examined in this study

Taxon 1	Taxon 2	*rbc*L (%)	*cox*1-5'(%)
Mesospora schmidtii	*M. schmidtii*	0 - 3.89	0 - 15.35
	M. elongata	6.39 - 8.72	19.37 - 21.91
	M. negrosensis	6.70 - 8.30	18.48 - 22.45
	Mesospora sp. C	7.13 - 8.49	18.33 - 21.16
	Mesospora sp. D	7.28 - 7.90	18.18 - 21.16
	Mesospora sp. 1	6.15 - 7.78	18.48 - 22.65
	Mesospora sp. 2	6.85 - 7.61	20.42 - 22.79
	"Mesospora" sp. 3	10.10 - 11.49	32.79 - 36.36
	Mesospora sp. G	10.92 - 11.66	-
M. elongata	*M. elongata*	0 - 0.82	0 - 0.6
	M. negrosensis	7.14 - 7.91	19.97 - 21.91
	Mesospora sp. C	7.28 - 7.91	21.16 - 21.76
	Mesospora sp. D	8.76 - 9.32	20.00- 21.01
	Mesospora sp. 1	7.20 - 7.68	21.01 - 22.65
	Mesospora sp. 2	6.98 - 7.28	19.08 - 19.37
	"Mesospora" sp. 3	10.63 - 11.26	34.58 - 35.47
	Mesospora sp. G	11.24 - 11.45	-
M. negrosensis	*M. negrosensis*	0 - 3.21	0 - 15.05
	Mesospora sp. C	5.57- 6.46	18.03 - 20.86
	Mesospora sp. D	6.40 - 7.38	18.50 -21.46
	Mesospora sp. 1	4.32 - 6.92	17.29 - 22.37
	Mesospora sp. 2	6.90 - 7.47	21.01 - 22.95
	"Mesospora" sp. 3	9.80 - 11.22	34.28 - 37.11
	Mesospora sp. G	10.10 - 10.56	-
Mesospora sp. C	*Mesospora* sp. C	0 - 1.49	0 - 0.45
	Mesospora sp. D	6.98 - 7.53	17.88 - 18.93
	Mesospora sp. 1	5.57- 7.20	17.14 - 18.33
	Mesospora sp. 2	7.28 - 7.61	20.57 - 20.86
	"Mesospora" sp. 3	10.77 - 11.57	34.87 - 36.96
	Mesospora sp. G	11.37 - 11.59	-
Mesospora sp. D	*Mesospora* sp. D	0 - 1.19	0.15 - 2.84
	Mesospora sp. 1	6.24 - 7.83	19.54 - 20.57
	Mesospora sp. 2	7.50 - 8.25	21.01 - 21.76
	"Mesospora" sp. 3	10.58 - 11.74	34.75 - 36.22

Table 4.1 (continued)

Taxon 1	Taxon 2	*rbc*L (%)	*cox*1-5'(%)
Mesospora sp. D	*Mesospora* sp. G	11.41 - 11.83	-
Mesospora sp. 1	*Mesospora* sp. 1	0 - 3.79	0 - 14.90
	Mesospora sp. 2	6.16 - 7.39	19.52 - 19.97
	"Mesospora" sp. 3	9.50 - 10.90	33.83 - 36.07
	Mesospora sp. G	10.03 - 10.85	-
Mesospora sp. 2	*Mesospora* sp. 2	0 - 0.15	0
	"Mesospora" sp. 3	9.87 - 10.85	34.28 - 35.02
	Mesospora sp. G	10.55- 10.79	-
"Mesospora" sp. 3	*"Mesospora"* sp. 3	0 - 3.71	0 - 14.75
	Mesospora sp. G	8.69 - 9.02	-
Diplura simplex	*Diplura* sp. B	3.36	0.15
	Diplura sp. C	2.99	-
	Diplura sp. 1	14.13 - 14.44	16.99 - 17.44
	Diplura sp. 2	14.78 - 15.04	16.54- 16.69
	Diplura sp. 3	15.57 - 16.10	19.90 - 21.16
	Diplura sp. 4	16.58 - 17.28	16.10
Diplura sp. B	*Diplura* sp. C	5.27	-
	Diplura sp. 1	16.33 - 16.77	16.84 - 17.29
	Diplura sp. 2	16.64 - 16.91	16.39 - 16.54
	Diplura sp. 3	17.62 - 18.41	19.75 - 21.01
	Diplura sp. 4	17.82 - 18.76	16.10
Diplura sp. C	*Diplura* sp. 1	12.77 - 13.21	-
	Diplura sp. 2	12.97 - 13.19	-
	Diplura sp. 3	14.70 - 15.15	-
	Diplura sp. 4	13.96 - 14.10	-
Diplura sp. 1	*Diplura* sp. 1	0 - 0.23	0 - 1.19
	Diplura sp. 2	7.90 - 8.20	13.26 - 14.16
	Diplura sp. 3	15.22 - 15.66	19.37 - 20.42
	Diplura sp. 4	9.58 - 10.02	12.67 - 13.26
Diplura sp. 2	*Diplura* sp. 2	0 - 0.30	0 - 0.15
	Diplura sp. 3	14.73 - 15.06	19.52 - 20.27
	Diplura sp. 4	10.80 - 11.47	14.01 - 14.16
Diplura sp. 3	*Diplura* sp. 3	0 - 0.53	0 - 2.69
	Diplura sp. 4	15.37 - 15.82	19.52 - 19.83
Diplura sp. 4	*Diplura* sp. 4	0 - 0.27	0
Ralfsia fungiformis	*Ralfsia* sp. B	9.75	-
	Ralfsia sp. C	10.46	-
	Ralfsia sp. 1	9.68	23.70
Ralfsia sp. B	*Ralfsia* sp. C	3.57	-
	Ralfsia sp. 1	0.07	-
Ralfsia sp. C	*Ralfsia* sp. 1	3.57	-
Ralfsia sp. 1	*Ralfsia* sp. 1	0	Only 1 sequence
Neoralfsia expansa	*Neoralfsia expansa*	0 - 2.70	0 - 9.39
Unidentified crustose brown species 1	Unidentified crustose brown species 1	0 - 0.30	0 - 0.3

4.2.1.1 Maximum likelihood analysis based on *rbc*L gene

The ML phylogenetic tree inferred using the *rbc*L data set (Fig. 4.1) showed that the crustose brown algae are not monophyletic but are nested in two distantly related clades (excluding *N. tingitanum*, *P. roscoffense* and *H. fluviatilis*). Clade 1 which correspond to the order Ralfsiales comprising the families Mesosporaceae, Neoralfsiaceae, and Ralfsiaceae; a possible new family and a subclade consisting of *Ralfsia*-like species, was resolved with strong bootstrap support (BP: 99%). Species of *Mesospora* excluding "*Mesospora*" sp. 3 were monophyletic and resolved with a strong bootstrap support of 99%. However, the family Mesosporaceae which include *H. saxigenum* in addition to *Mesospora* spp. was only moderately supported (BP: 73%). Each of the six *Mesospora* spp. subclades was resolved with maximum BP, except for *Mesospora* sp. 1 (BP: 96%). The monotypic family Neoralfsiaceae was fully supported (BP: 100%) and formed a non-supported sister relationship with the subclade consisting of "*Mesospora*" sp. 3 and *Mesospora* sp. G. "*Mesospora*" sp. 3 shared a close relationship with *Mesospora* sp. G with 99% BP and may constitute a new family of its own. The unidentified crustose brown alga species 2 did not group with any known taxa. The Ralfsiaceae and the subclade consisting of *Ralfsia*-like species were both strongly supported by 98% BP and 100% BP, respectively.

Clade 2 was resolved as a polytomy and consisted of *Diplura* spp., the order Ishigeales, two brown crusts of uncertain taxonomic placement (*Petroderma maculiforme* and *Porterinema fluviatile*) and the unidentified crustose brown alga species 1. *Diplura* spp. were monophyletic, but with a weak support of 56% BP. However, this increased to 99% BP when *Diplura* sp. 3 was omitted. *Diplura* spp. were sister to the Ishigeales, albeit without any support. The unidentified crustose brown alga species 1 formed an unsupported relationship with *P. fluviatile*.

The monophyly of all the brown algal orders was strongly supported (BP: 99%). Members of Clade 2 diverged after the earliest phaeophycean order, the Discosporangiales and the Sphacelariales-Syringodermatales-Dictyotales-Onslowiales (SSDO) group. Meanwhile, Clade 1 or the Ralfsiales is a more recent lineage resolved as part of the 'brown algal crown radiation' or BACR, a group which includes most currently recognised brown algal orders.

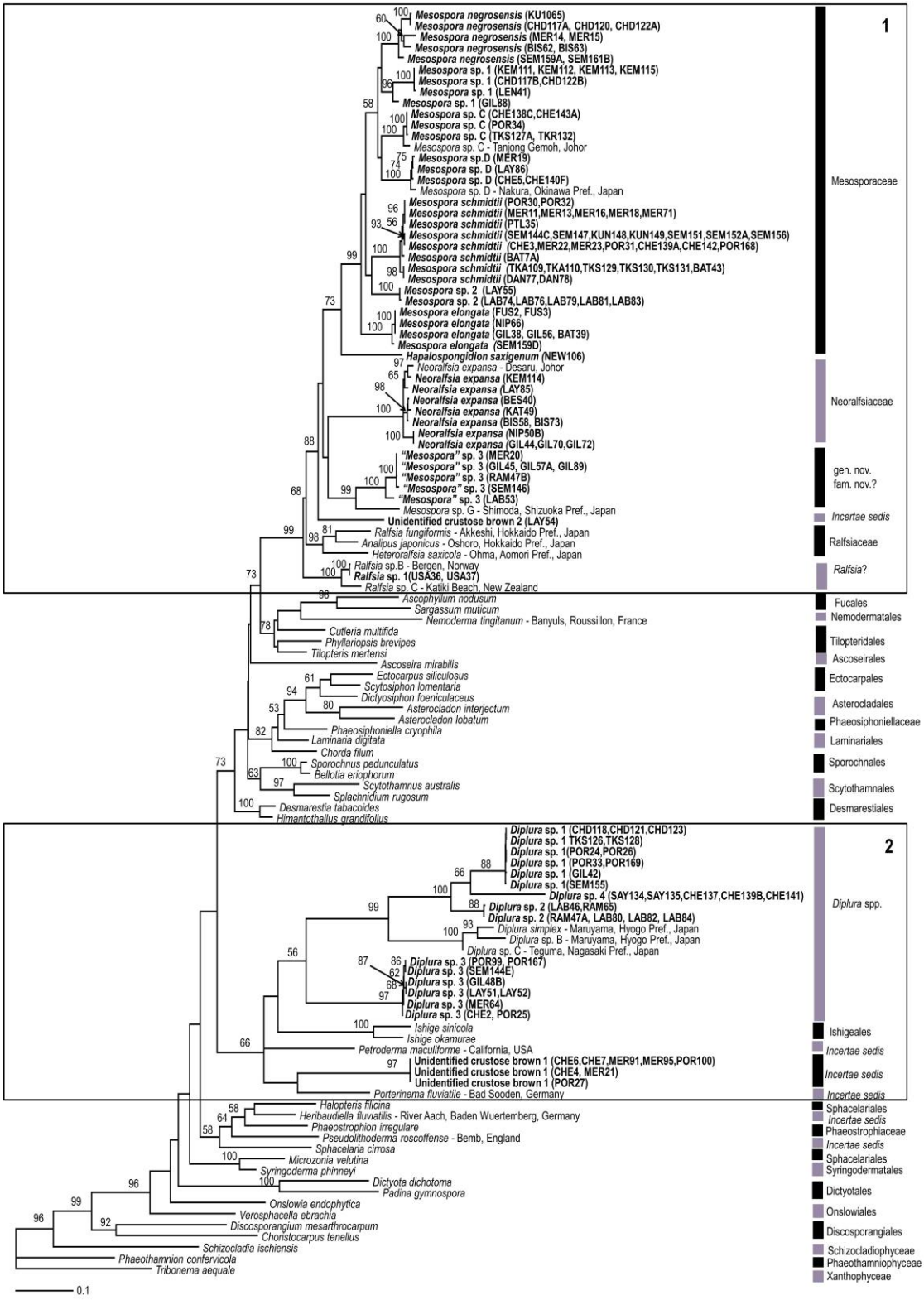

Figure 4.1 ML phylogeny inferred based on the *rbc*L data set. Numbers above each branch denote bootstrap percentage (BP) and branches without value indicate percentages of <50%. Scale bar = 0.1 substitution per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa.

4.2.1.2 Maximum parsimony analysis based on *rbc*L gene

The crustose brown algae are shown to be polyphyletic and split into three major clades (excluding *N. tingitanum*, *P. roscoffense* and *H. fluviatilis*) as shown in Fig. 4.2. Clade 1 correspond to the order Ralfsiales and comprised the families Mesosporaceae, Neoralfsiaceae, and Ralfsiaceae; a possible new family and a subclade consisting *Ralfsia*-like species, and was resolved with maximum bootstrap support. Species of *Mesospora* excluding "*Mesospora*" sp. 3 were monophyletic and resolved with strong BP of 94%. Nevertheless, the family Mesosporaceae inclusive of *H. saxigenum* was only weakly supported (BP: 59%). Each of the six *Mesospora* spp. subclades was resolved with maximum BP, except for *Mesospora* sp. 1 (BP: 92%). "*Mesospora*" sp. 3 was resolved in a subclade with *Mesospora* sp. G and this relationship was strongly supported (BP: 96%). The family Neoralfsiaceae was fully supported (BP: 100%) and formed a non-supported relationship with the unidentified crustose brown alga species 2. The family Ralfsiaceae was moderately supported (BP: 87%) whereas the subclade comprising *Ralfsia*-like species was fully supported.

The unidentified crustose brown alga species 1 formed a weakly supported relationship (BP: 57%) with *P. fluviatile* in Clade 2 and the two were sister to a clade comprising the Ishigeales and *P. maculiforme*. Clade 3 consisted of *Diplura* spp. which were sister to the Dictyotales, nonetheless this relationship was not supported. The *Diplura* spp. were monophyletic, but with a moderate support of 70% BP. The exclusion of *Diplura* sp. 3 however, lead to a higher BP of 96%. The monophyly of all the brown algal orders was strongly supported (BP: 96%). In this analysis, the SSDO group were not monophyletic but split into two in which the Syringodermatales-Onslowiales-Sphacelariales grouped with Clade 2 while the Dictyotales grouped with Clade 3. Clade 1 was again resolved as part of the BACR group.

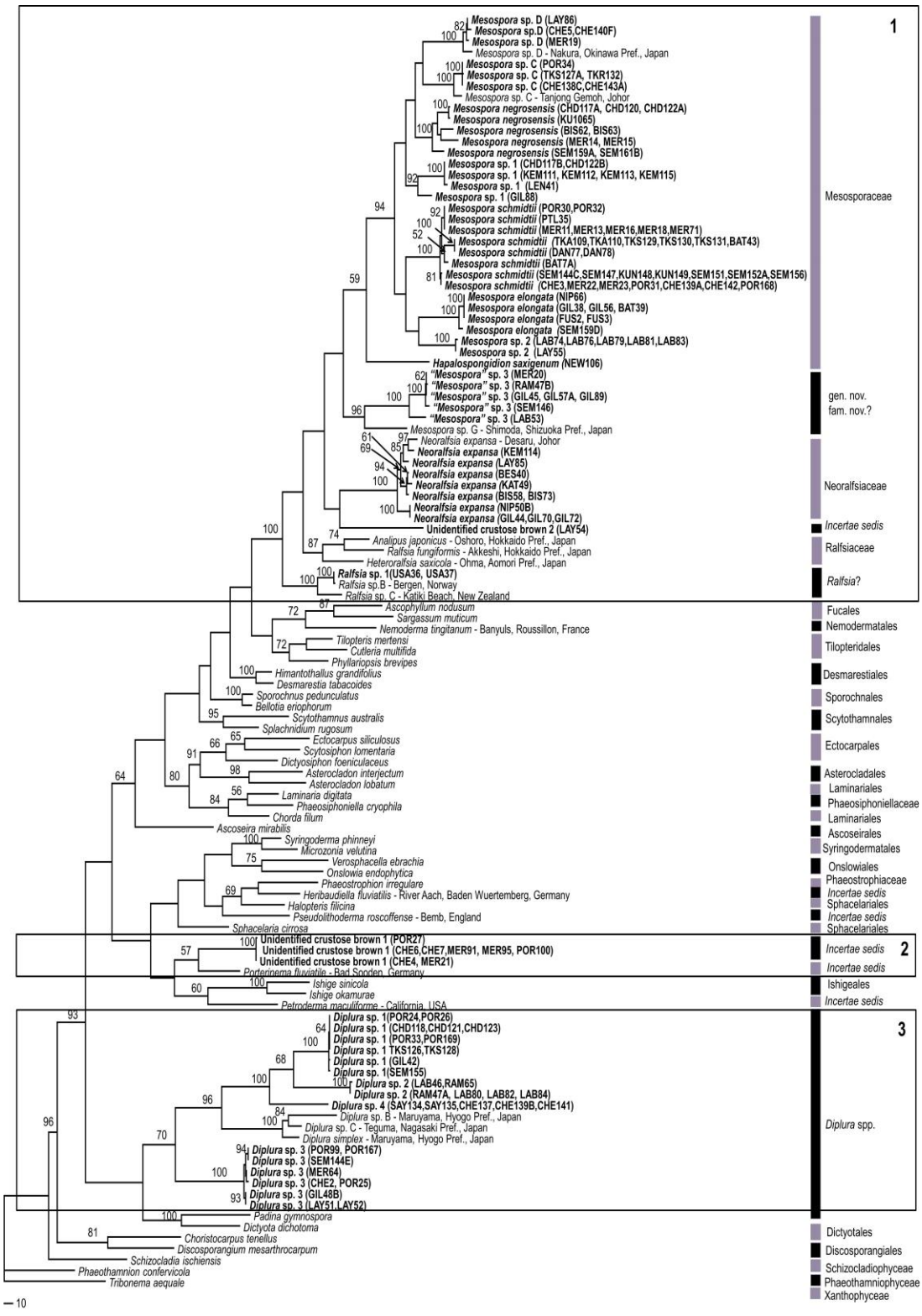

Figure 4.2 One of the 17 most parsimonious tree inferred based on the *rbc*L data set. Numbers above each branch denote bootstrap percentage (BP) and branches without value indicate percentages of <50%. Scale bar = 10 substitutions per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa.

4.2.1.3 Bayesian inference analysis based on *rbc*L gene

Figure 4.3 summarised the results of this analysis. The crustose brown algae were not monophyletic but instead distributed into two major clades (*N. tingitanum, P. roscoffense* and *H. fluviatilis* excluded). Clade 1 represented the order Ralfsiales and comprised the families Mesosporaceae, Neoralfsiaceae, and Ralfsiaceae; a possible new family and a subclade consisting of *Ralfsia*-like species, and was resolved with maximum support (PP: 1.00). The monophyly of *Mesospora* spp. (excluding the putative new genus, "*Mesospora*" sp. 3) and the family Mesosporaceae were fully supported (PP: 1.00). Each of the seven *Mesospora* spp. subclades was resolved with maximum support (PP: 1.00). "*Mesospora*" sp. 3 and the closely related *Mesospora* sp. G formed a weakly supported (PP: 0.61) relationship with the Neoralfsiaceae which itself was fully supported (PP: 1.00). The unidentified crustose brown alga species 2 did not clade with any known taxa. The Ralfsiaceae and the subclade consisting of *Ralfsia*-like species were also fully supported (PP: 1.00).

In Clade 2, a polytomy of three lineages was obtained. The first lineage, resolved with a weak PP of 0.86 comprised species of *Diplura.* Aside from *Diplura* sp. 3, the monophyly of the rest of *Diplura* spp. was fully supported (PP: 1.00). The unidentified crustose brown alga species 1 formed a strongly supported relationship (PP: 0.99) with *P. fluviatile* in the second lineage. The third lineage comprising the Ishigeales and *P. maculiforme* was resolved with a weak support (PP: 0.81). The monophyly of all the brown algal orders was fully supported (PP: 1.00). Members of Clade 2 diverged early after the Discosporangiales and before the SSDO and BACR groups. Clade 1 was resolved as part of the BACR entity.

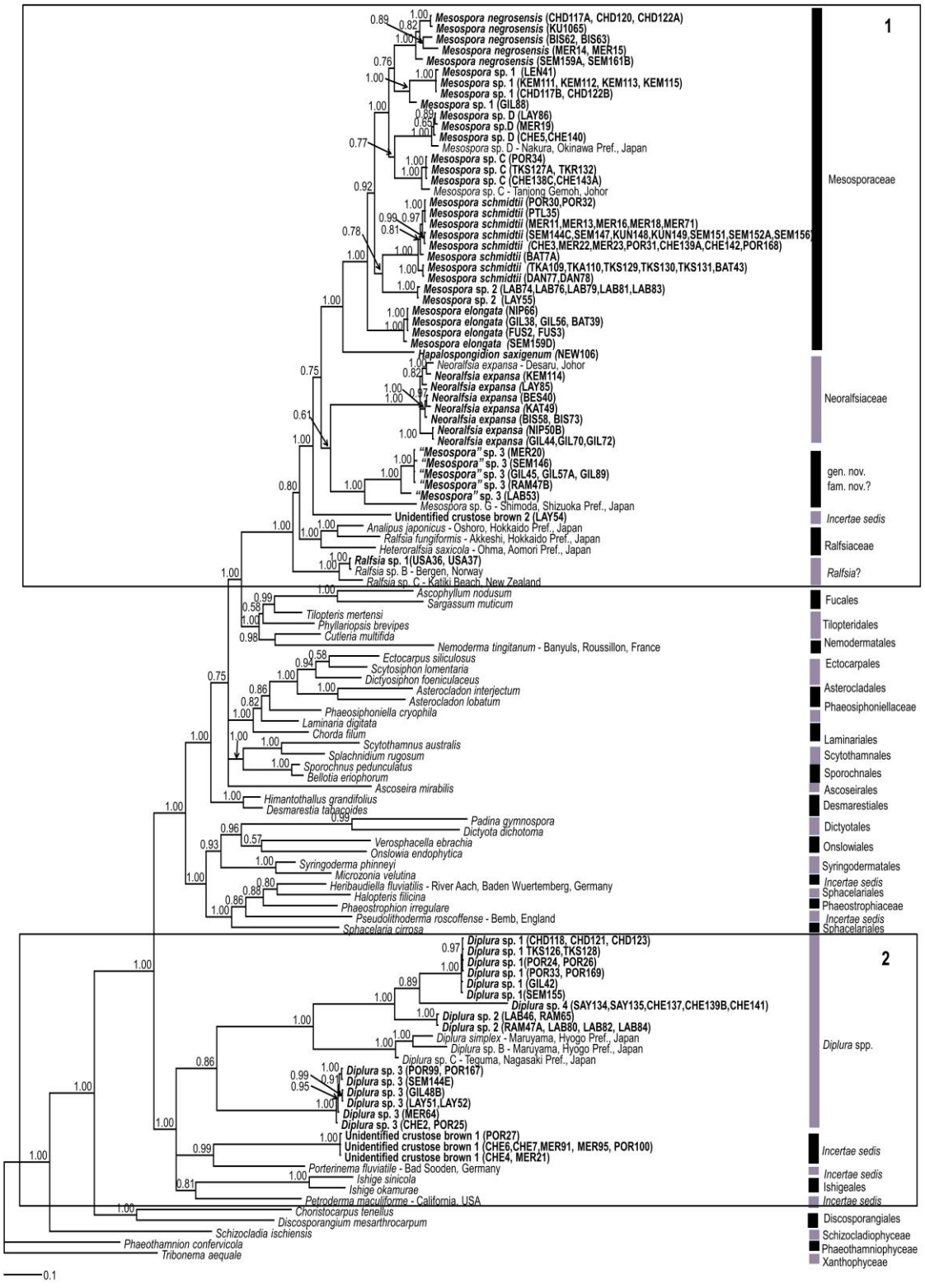

Figure 4.3 BI phylogeny inferred based on the *rbc*L data set. Numbers above each branch denote posterior probabilities (PP) and branches without value indicate probabilities of <0.50. Scale bar = 0.1 substitution per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa.

4.2.2 Analysis of *cox*1-5' gene sequence data set

For the *cox*1-5' only data set, the alignment consisted of 677 characters of which 390 (58%) sites were variable and 362 (54%) were parsimony informative. The number of parsimonious trees obtained was 3,474, the tree length was 2,902, and CI and RI indices were 0.2567 and 0.7371, respectively. The intraspecific divergences (Table 4.1) ranged from 0 – 0.15% in *Diplura* sp. 2 to 0 - 15.35% in *M. schmidtii* (Appendix D). There were no intraspecific divergences within samples of *Diplura* sp. 4 and *Mesospora* sp. 2. Within *Diplura*, interspecific divergences ranged from 12.67 – 13.26% between *Diplura* sp. 1 and *Diplura* sp. 4 to 19.90 – 21.16% between *D. simplex* and *Diplura* sp. 3. The genetic variation between *Diplura* sp. B and *Diplura simplex* was only 0.15%. Within *Mesospora*, interspecific divergences ranged from 17.14 – 18.33% between *Mesospora* sp. C and *Mesospora* sp. 1 to 21.01 – 22.95% between *M. negrosensis* and *Mesospora* sp. 2. The genetic variation between the putative new genus ("*Mesospora"* sp. 3) and the seven species of *Mesospora* ranged from 32.79% to 37.11%. Pairwise distance between *Ralfsia* sp. 1 and *R. fungiformis* was 23.70%. The partial *cox*1 gene was successfully sequenced for 157 specimens (95% success). There was no alignment problem for this data set.

4.2.2.1 Maximum likelihood analysis based on *cox*1-5' gene

The phylogenetic tree in Fig. 4.4 showed that the crustose brown algae split into three main clades. Clade 1 was unsupported and consisted of the Mesosporaceae, Neoralfsiaceae, Ralfsiaceae; *Ralfsia* sp. 1; the unidentified crustose brown alga species 2; *P. roscoffense* and a possible new family. The putative new genus, "*Mesospora*" sp. 3 surprisingly grouped within the Mesosporaceae albeit with a long terminal branching and a moderate BP of 71%. Each of the *Mesospora* spp. subclade was resolved with weak to strong bootstrap support: *Mesospora* sp. C (94%), *Mesospora* sp. 1 (71%), *M. elongata* (74%) and *Mesospora* sp. 2 (67%), *M. schmidtii* (73%), *Mesospora* sp. D (98%) and *M. negrosensis* (79%). The monophyly of *Mesospora* spp. (including "*Mesospora*" sp. 3) was weakly supported (BP: 62%). The brown crust *P. roscoffense* unexpectedly grouped within Clade 1 and was sister to species of *Mesospora*. The Ralfsiaceae and a subclade consisting of the *Ralfsia*-like species and the unidentified crustose brown alga species 2 were resolved without support while the Neoralfsiaceae was resolved with a strong bootstrap support (BP: 95%). Species of *Diplura* were monophyletic and nested in Clade 2 which was resolved without support. *Diplura* sp. 3 was resolved with a strong support (BP: 93%) and was separated from the rest of the *Diplura* spp. which grouped together with moderate support (BP: 70%). Clade 3 was represented by the unidentified crustose brown alga species 1, resolved with strong support (BP: 95%).

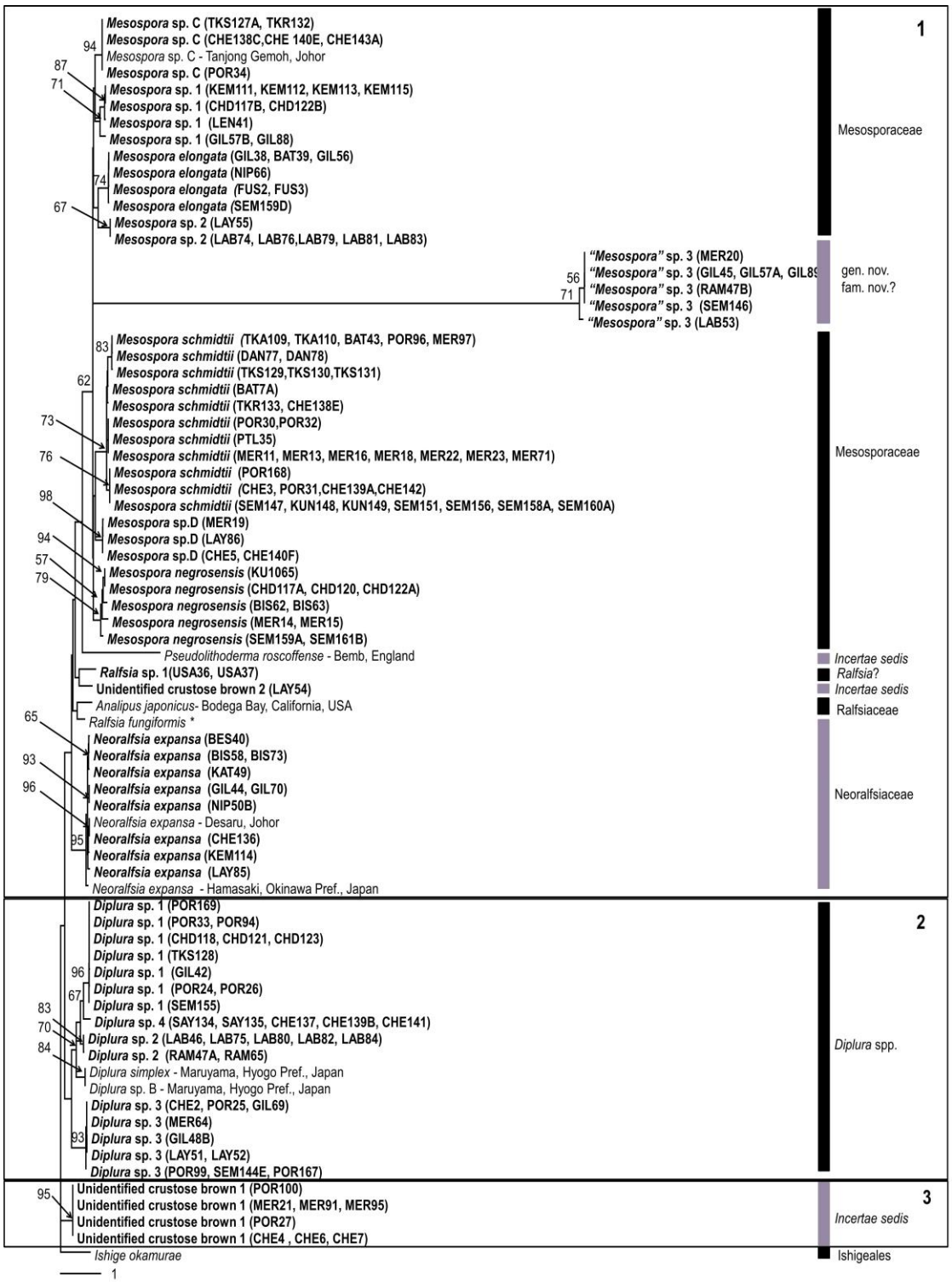

Figure 4.4 ML phylogeny inferred based on the *cox*1-5' data set. Numbers above each branch denote bootstrap percentage (BP) and branches without value indicate percentages of <50%. Scale bar = 1 substitution per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa, * origin undetermined.

4.2.2.2 Maximum parsimony analysis based on *cox*1-5' gene

The crustose brown algae were again split into three main clades as shown in Fig. 4.5. Clade 1 comprised the Mesosporaceae, Neoralfsiaceae, Ralfsiaceae; a *Ralfsia*-like species; the unidentified crustose brown alga species 1 and 2 and a possible new family. *Mesospora* spp. of the Mesosporaceae were monophyletic with a moderate bootstrap support of 78%. Each of the *Mesospora* spp. subclade was resolved with full bootstrap support (BP: 100%) except for *Mesospora* sp. 1 (BP: 92%). The putative new genus, "*Mesospora*" sp. 3 was sister to the Mesosporaceae but this relationship was unsupported. The Neoralfsiaceae and the unidentified crustose brown alga species 1 were each resolved with maximum support (BP: 100%) while the Ralfsiaceae, unidentified crustose brown alga species 2 and *Ralfsia* sp. 1 were grouped in an unsupported subclade. Species of *Diplura* (excluding *Diplura* sp. 3) were monophyletic and were distributed in Clade 2 which was resolved with moderate support (BP: 82%). *Diplura* sp. 3 diverged earlier on its own in Clade 3 and was resolved with full support (BP: 100%). *Pseudolithoderma roscoffense* did not group with any of the other crustose brown algae.

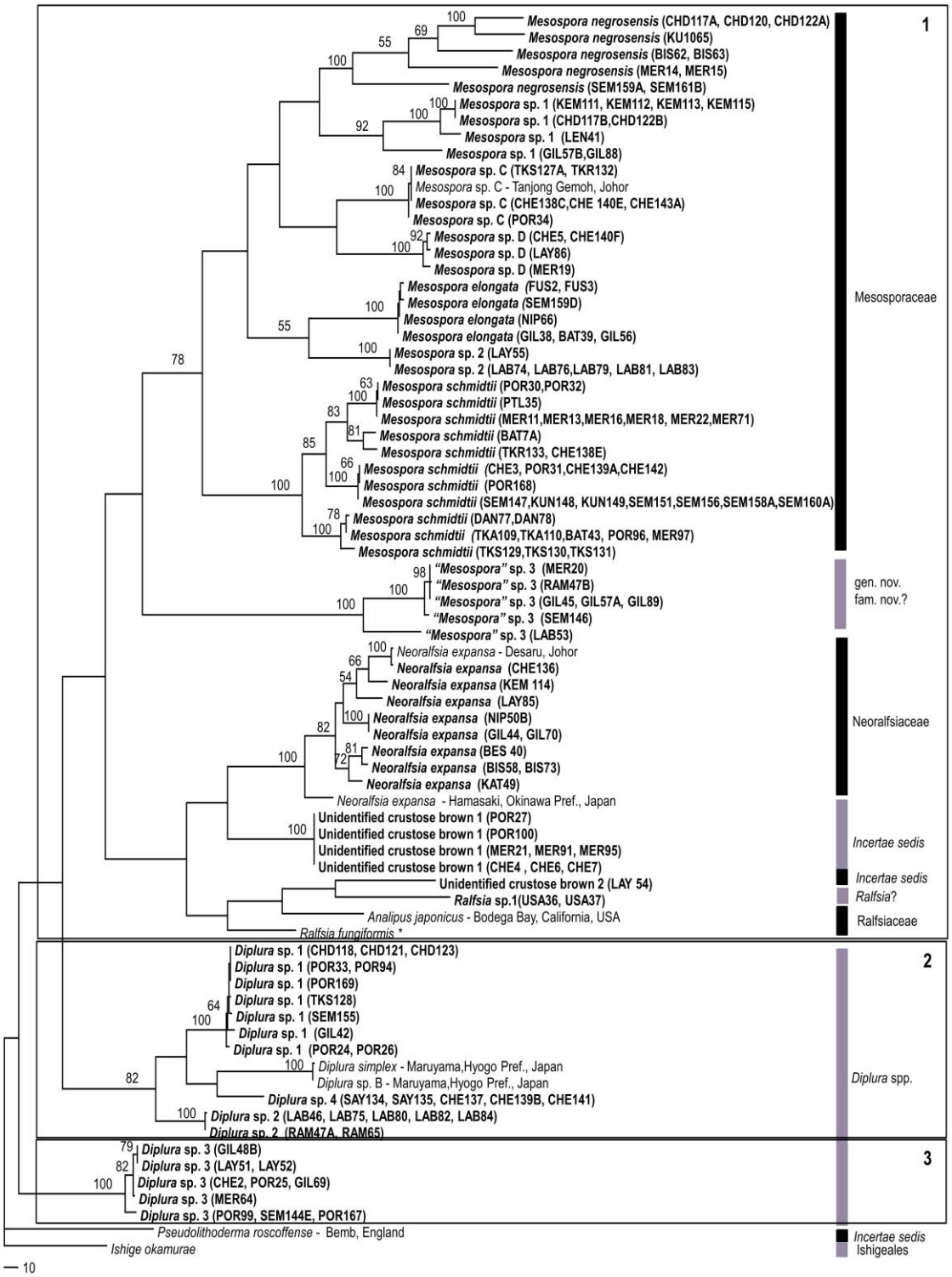

Figure 4.5 One of the 3,474 most parsimonious tree inferred based on the *cox*1-5' data set. Numbers above each branch denote bootstrap percentage (BP) and branches without value indicate percentages of <50%. Scale bar = 10 substitutions per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa, * origin undetermined.

4.2.2.3 Bayesian inference analysis based on *cox*1-5' gene

The crustose brown algae were partially resolved and three main clades were observed (Fig. 4.6). The families Mesosporaceae, Neoralfsiaceae and Ralfsiaceae; *Ralfsia* sp. 1; unidentified crustose brown alga species 2 and *P. roscoffense* were grouped in Clade 1 (PP: 0.99), species of *Diplura* in Clade 2 (PP: 1.00) and the unidentified crustose brown alga species 1 in Clade 3 (PP: 1.00). Each of the *Mesospora* spp. subclade were resolved with maximum support (PP: 1.00) with the exception of *Mesospora* sp. 1 (PP: 0.99). The Mesosporaceae was resolved with a weak support (PP: 0.52). *Mesospora* sp. 3 formed a moderately supported (PP: 0.93) sister relationship to the Mesosporaceae with an exceptionally long terminal branching. *Pseudolithoderma roscoffense* did not group with any taxa while the unidentified crustose brown alga species 2 was sister to *Ralfsia* sp. 1 with a weak support (PP: 0.52). The Neoralfsiaceae and Ralfsiaceae were each resolved with a full (PP: 1.00) and strong (PP: 0.96) support, respectively.

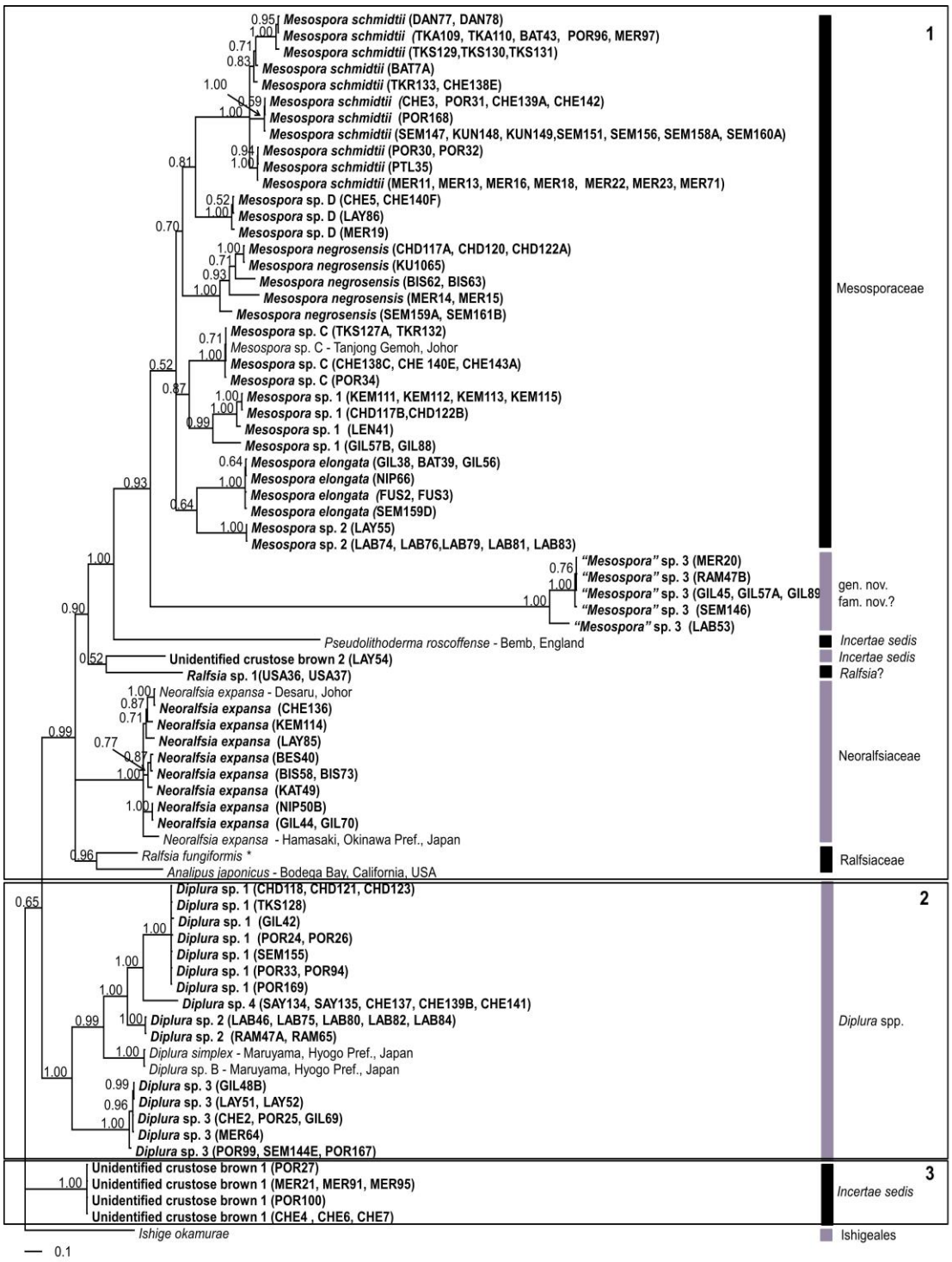

Figure 4.6 BI phylogeny inferred based on the *cox*1-5' data set. Numbers above each branch denote posterior probabilities (PP) and branches without value indicate probabilities of <0.50. Scale bar = 0.1 substitution per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa, * origin undetermined.

4.2.3 Analysis of concatenated *rbc*L-*cox*1-5' gene sequence data set

Alignment of the concatenated *rbc*L-*cox*1-5' gene sequences consisted of 2,017 nucleotides of which 1,251 (62%) nucleotides were variable and 1,070 (53%) sites were parsimony-informative. One most parsimonious tree was obtained, and tree length was 10,281, the CI index was 0.2182 and the RI index was 0.5009.

4.2.3.1 Maximum likelihood analysis based on combined *rbc*L and *cox*1-5' gene sequences

The crustose brown algae were shown to be polyphyletic and distributed in two main clades (exclusive of *N. tingitanum*, *H. fluviatilis* and *P. roscoffense*) as shown in Fig. 4.7. Clade 1 which was resolved with strong support (BP: 99%) identifies with the Ralfsiales and consisted of the Mesosporaceae, Neoralfsiaceae, Ralfsiaceae, a subclade representing a possible new family ("*Mesospora*" sp. 3 and *Mesospora* sp. G), unidentified crustose brown alga species 2 and a subclade consisting of *Ralfsia*-like species. The Mesosporaceae which included seven *Mesospora* spp. and *H. saxigenum* was resolved with a moderate BP of 77% and each of the *Mesospora* spp. subclade was resolved with full support (BP: 100%). "*Mesospora*" sp. 3 and its sister taxon, *Mesospora* sp. G grouped together with strong support (BP: 99%). The unidentified crustose brown alga species 2 grouped with Neoralfsiaceae but without support. The Neoralfsiaceae and the clade comprising *Ralfsia*-like species were both resolved with maximum support (BP: 100%). Meanwhile, the Ralfsiaceae was resolved with moderate support (BP: 74%).

Near the basal end of the phylogenetic tree, species of *Diplura*, the Ishigeales, *P. maculiforme*, unidentified crustose brown alga species 1 and *P. fluviatile* formed a

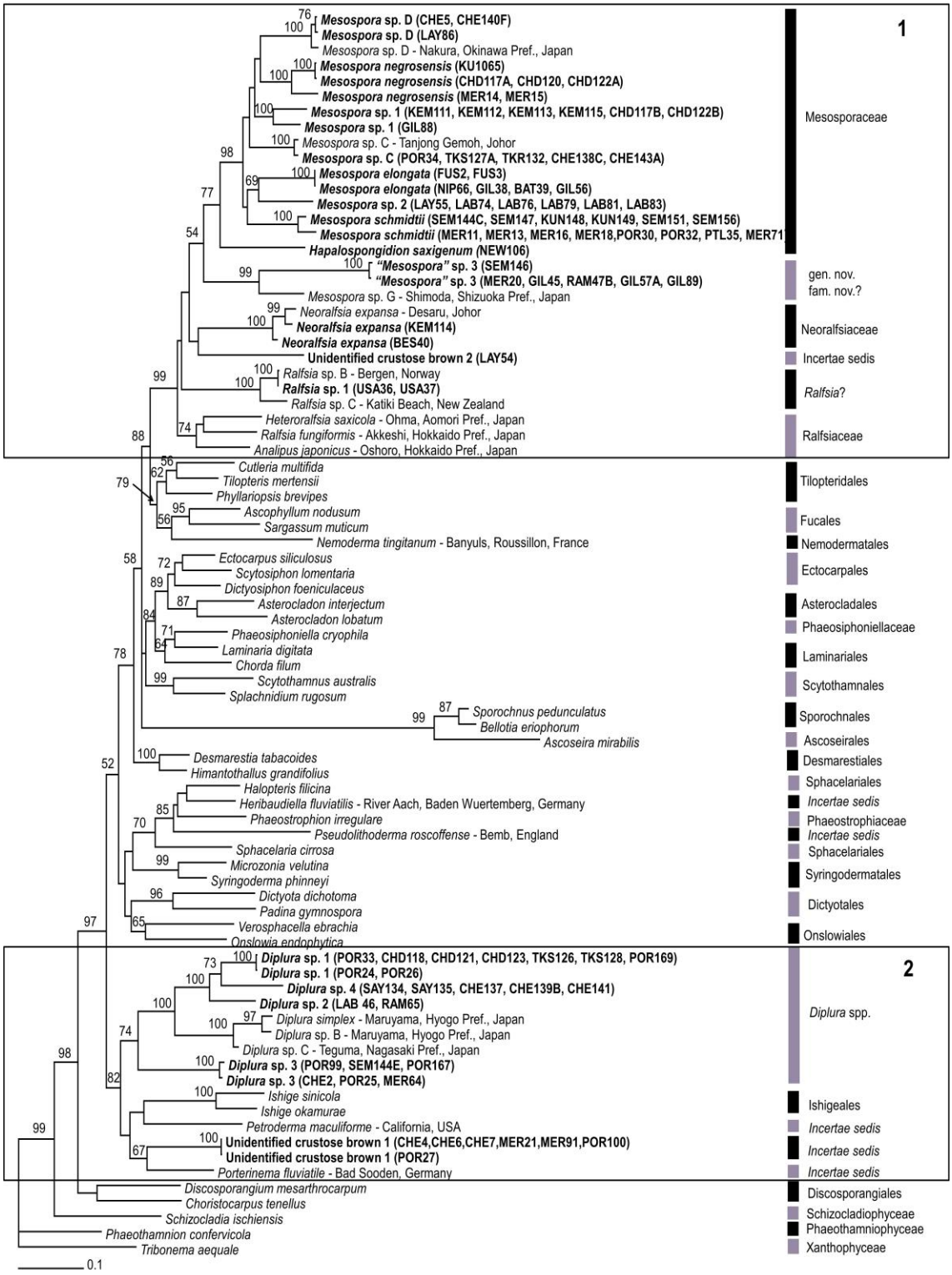

Figure 4.7 ML phylogeny inferred based on the combined *rbc*L and partial *cox*1 data set. Numbers above each branch denote bootstrap percentage (BP) and branches without value indicate percentages of <50%. Scale bar = 0.1 substitution per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa.

moderately supported (BP: 82%) clade of their own i.e. Clade 2, and diverged early after the Discosporangiales. Clade 2 split into two subclades, the first subclade consisting of monophyletic *Diplura* spp. with moderate support (BP: 74%) and the second subclade comprised the fully supported Ishigeales; its unsupported sister taxon, *P. maculiforme* and the unidentified crustose brown alga species 1 with its weakly supported (BP: 67%) sister taxon, *P. fluviatile*. The Discosporangiales was the earliest brown algae to diverge followed by members of Clade 2, the SSDO group and subsequently the BACR groups which included the Ralfsiales.

4.2.3.2 Maximum parsimony analysis based on combined *rbc*L and *cox*1-5' gene sequences

The crustose brown algae were again polyphyletic and nested in two major clades (Fig. 4.8). Members of the Ralfsiales were distributed in Clade 1 and resolved with a strong BP of 99%. The monophyly of the Mesosporaceae was unsupported, although species of *Mesospora* grouped with strong support (BP: 90%). Each of the *Mesospora* spp. subclades was resolved with maximum support (BP: 100%). The subclade consisting "*Mesospora*" sp. 3 and *Mesospora* sp. G was resolved with strong support (BP: 97%). The Neoralfsiaceae and the subclade consisting *Ralfsia*-like species were both fully supported (BP: 100%) and the unidentified crustose brown alga species 2 was sister to the latter but without support. The Ralfsiaceae was resolved with moderate support (BP: 76%). In Clade 2 which was resolved without support, *Diplura* spp. were monophyletic albeit with weak support (BP: 64%) and formed a non-supported sister relationship with the Ishigeales and *P. maculiforme*. The unidentified crustose brown alga species 1 was sister to *P. fluviatile*. Members of Clade 2 were among the earliest diverging phaeophyceaen lineages after the Discosporangiales and prior to the SSDO group.

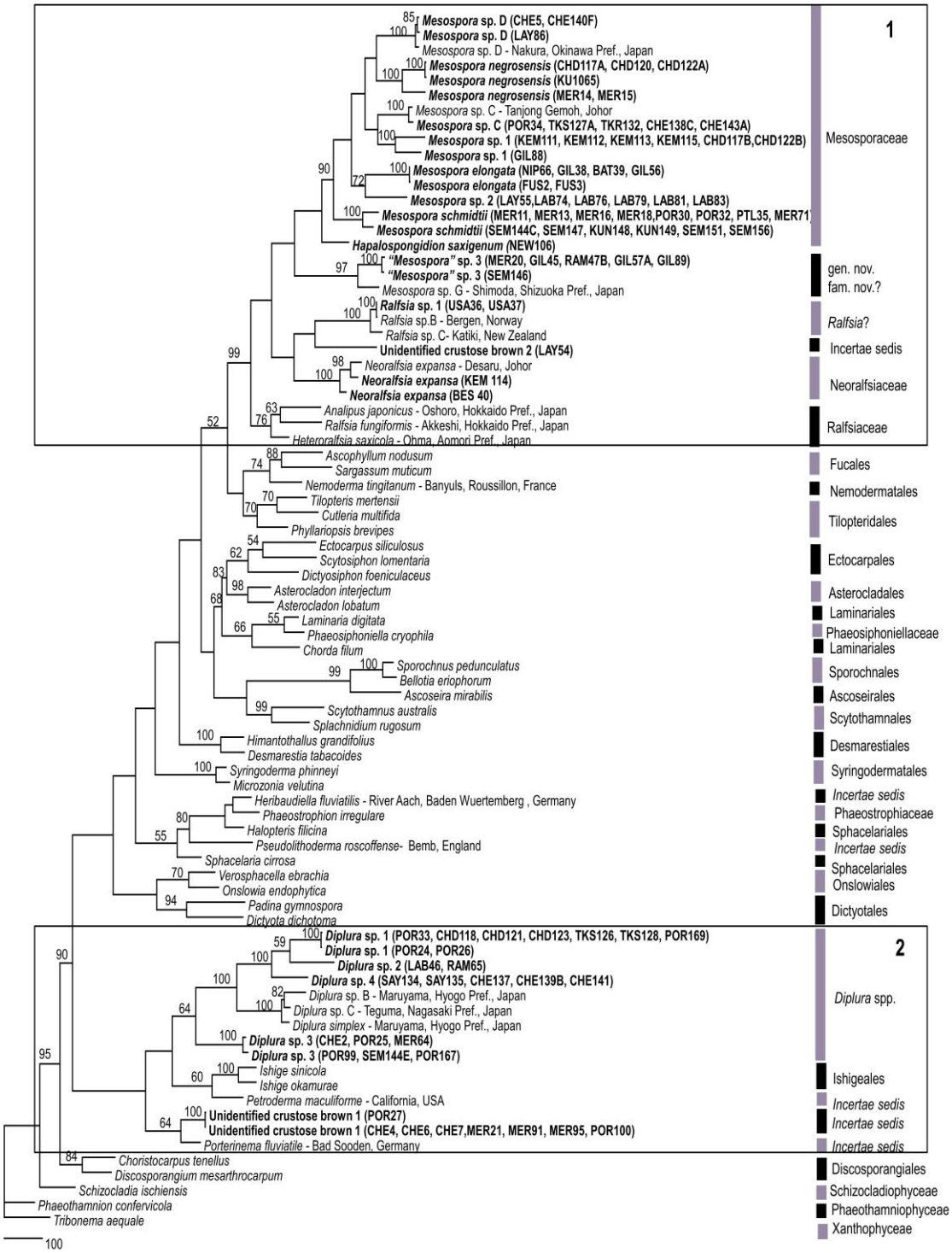

Figure 4.8 The single most parsimonious tree inferred based on the combined *rbc*L and partial *cox*1 data set. Numbers above each branch denote bootstrap percentage (BP) and branches without value indicate percentages of <50%. Scale bar = 100 substitutions per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa.

4.2.3.3 Bayesian inference analysis based on combined *rbc*L and *cox*1-5' gene sequences

A similar trend was observed in which the crustose brown algae split into two fully supported (PP: 1.00) major clades (Fig. 4.9), the first clade corresponded to the Ralfsiales and the early-diverging second clade comprised *Diplura* spp., the Ishigeales, *P. maculiforme*, *P. fluviatile* and the unidentified crustose brown alga species 1. In Clade 1, the families Neoralfsiaceae and Ralfsiaceae; the subclade consisting of *Ralfsia*-like species and the subclade comprising the putative new genus were all resolved with maximum PP of 1.00. The monophyly of the Mesosporaceae was strongly supported (PP: 0.99) and each of the *Mesospora* spp. subclade received full support (PP: 1.00). The unidentified crustose brown alga species 2 formed a moderately supported relationship (PP: 0.93) with the Neoralfsiaceae. Two subclades were observed in Clade 2 and they were both strongly supported (PP of first subclade: 1.00; PP of second subclade: 0.98). In the first subclade, species of *Diplura* were strongly monophyletic (PP: 1.00) while in the second subclade, the Ishigeales formed a strongly supported (PP: 0.99) sister relationship with *P. maculiforme* while the unidentified crustose brown alga species 1 was resolved as a sister to *P. fluviatile* (PP: 1.00). The Discosporangiales was the first brown algae to diverge followed by members of Clade 2, the SSDO group and subsequently the BACR groups which included the Ralfsiales.

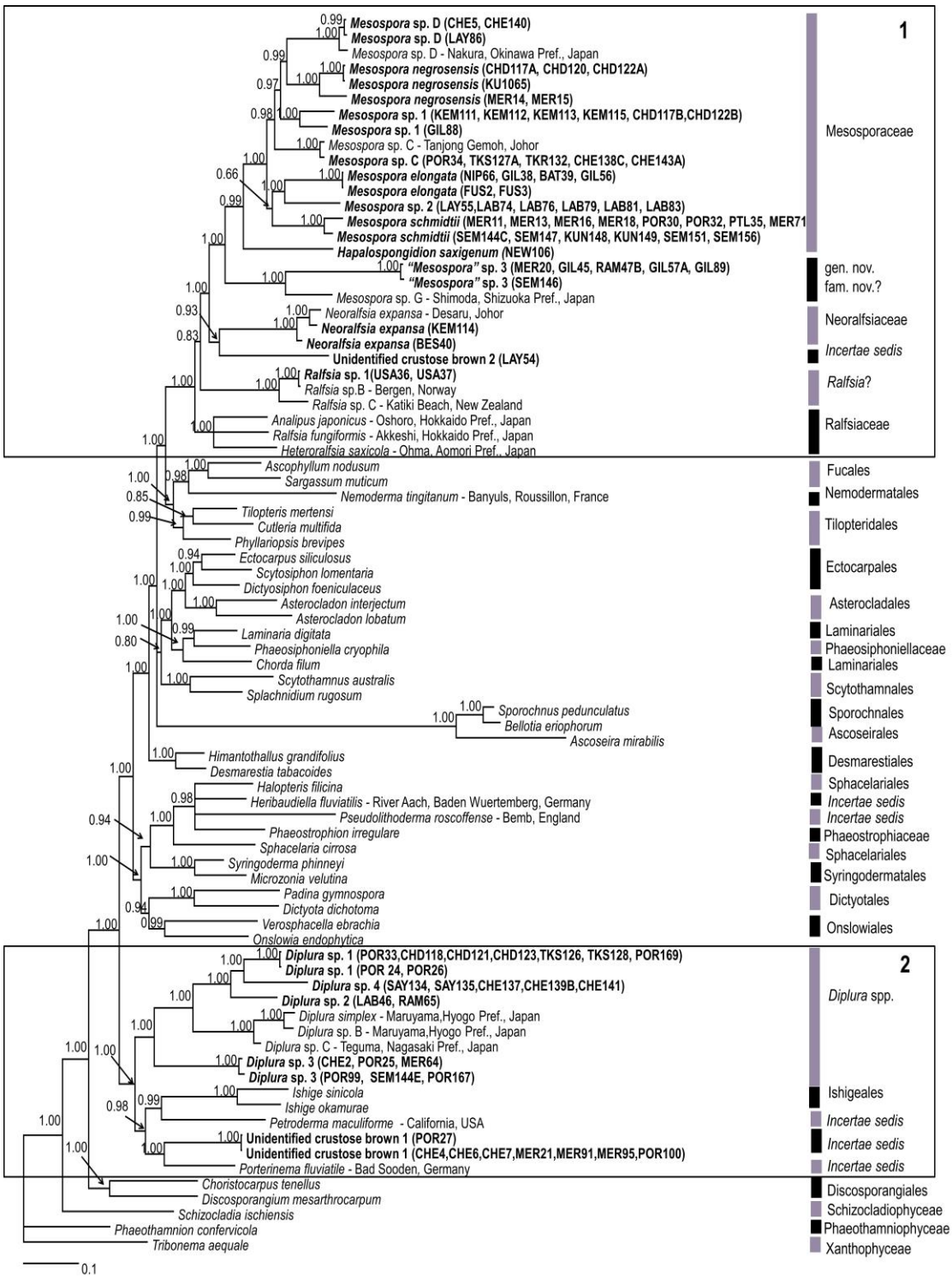

Figure 4.9 BI phylogeny inferred based on the combined *rbc*L and partial *cox*1 data set. Numbers above each branch denote posterior probabilities (PP) and branches without value indicate probabilities of <0.50. Scale bar = 0.1 substitution per site. Specimens of this study are highlighted in bold. Place of origin is given next to the crustose brown algal taxa.

4.3 Morphological and anatomical observations

The terms unilocular and plurilocular reproductive structures are used as the exact nature of the reproductive cells (i.e. gametes or spores) produced could not be established with certainty.

4.3.1 *Mesospora* spp.

The thalli of *Mesospora* are relatively thicker than *Diplura* and yet thinner than *Neoralfsia expansa*. Vegetative filaments are relatively loosely adherent (with exception in certain species) compared to *Diplura* and *N. expansa*. Plurilocular reproductive structures are more common than unilocular reproductive structures. Characters useful for species level identification include the number of sterile cells terminating the plurilocular bodies, and the position and number of stalk cells where the unilocular reproductive structures are inserted. A single chloroplast was present in each cell.

4.3.1.1 *Mesospora schmidtii* Weber-van Bosse, Fig. 4.10

The thallus were epilithic and formed smooth (at times, flaky), dark brown-black crusts; gelatinous (especially fertile crusts) upon contact with water (Figs. 4.10a - c). Thallus was firmly adherent to the rock substratum over the entire under surface, without rhizoids. The crust outline was discrete and orbicular in juvenile plants; often became irregular in older thalli as a result of confluence with surrounding thalli. In squash preparations, the thallus comprised a horizontal basal layer of one to four (generally two) layers of adjoined rectangular cells. The basal region gave rise to erect, free, simple and unbranched filaments that were composed of eight to 20 cells (Fig. 4.10d).

These loosely associated filaments, held within a gelatinous matrix, were readily separated under light pressure. The erect filaments were slightly clavate in appearance. Cells at the basal end of the filaments were cylindrical; measuring 6.7 - 14.8 (-17.7) μm in length and 2.8 - 6.8 (-10.0) μm in width; the length to diameter ratio was 1.2:1 to 3.5:1. The upper region cells were subglobose, and they were 5.2 - 10.7 (-13.2) μm in length and 4.3 - 8.8 (-11.2) μm in width; the length to diameter ratio was 0.6:1 to 2.5:1. Intercalary plurilocular reproductive structures occurred in the upper portion of erect filaments, terminated by two to three sterile terminal cells (Fig. 4.10e). Plurilocular bodies were 12.4 - 52.6 μm in length, 6.7 - 14.1 μm in width; the locules were somewhat regularly arranged. Unilocular structures were terminally inserted on stalks of up to four cells; they were lateral and basal to the parent erect filament and to the surrounding filaments (Fig. 4.10f). The unilocular structures were 24.2 - 143.2 μm in length and 14.7- 54.8 μm in diameter. The stalk cells were shorter than cells of the vegetative filaments. The two types of reproductive structures were not conclusively found on the same plant; it was difficult to separate confluent individuals because of the continuous crust. Infrequently, colourless hairs emerged as tufts from depressions in the basal layer. Sheath-like cell wall remnants were attached prominently above and/or below the plurilocular structures; occasionally sheath-like remnants were attached to sterile terminal cells and other vegetative cells.

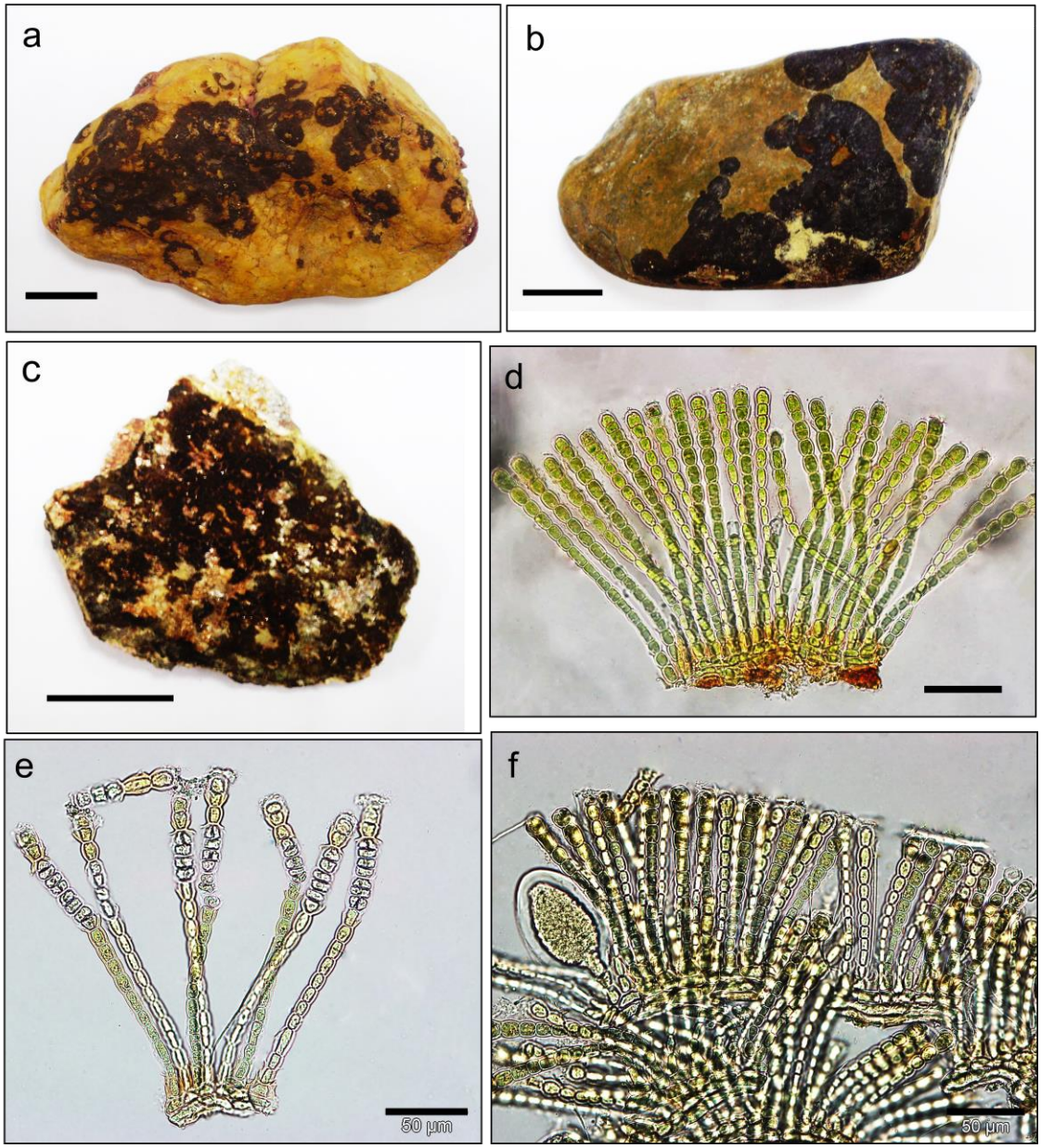

Figure 4.10 *Mesospora schmidtii* (a - c) Various morphologies of thalli on substratum [Voucher no. = PSM 12372, PSM 12348 and PSM 12201, respectively] Scale bars = 2 cm. (d) Vegetative filaments arising from a distromatic basal plate [Voucher no. = PSM 12235] (e) Plurilocular reproductive structures borne near the apex of the erect filaments and capped by two to three sterile terminal cells [Voucher no. = PSM 12207] (f) A unilocular reproductive structure terminally inserted on two stalk cells and basal-lateral to the erect filaments [Voucher no. = PSM 12207] Scale bars = 50 µm.

4.3.1.2 *Mesospora negrosensis* West & Calumpong, Fig. 4.11

Thallus thin and epilithic, dark brown to black, smooth to crusty, sometimes with an eroded centre and thus resemble ring-like crusts, circular in outline when young, later becoming irregular and often confluent (Figs. 4.11a – c). Thallus was generally adherent to the substratum by whole under surface except for those which form cracked and flaky crust, where the margins or eroded centre tended to be free. Rhizoids were not observed. Thallus especially fertile parts tended to be gelatinous upon contact with water. Two morphotypes were observed for this species, one is similar to the original description of this species from field-collected specimens and the other morphotype slightly resembled the original description of this species from cultured specimens. In the first morphotype, the erect filaments were laterally free throughout except for the thin basal layer (approximately 8 - 10 µm thick) which consisted horizontally bilayer cells, two to three times wider than long (Fig. 4.11d). The erect filaments were somewhat shorter, approximately 89.7 – 152.6 µm in length; consisting of nine cells (not inclusive of the sterile terminal cells and plurilocular bodies). The cells were 2.9 – 6.5 µm broad and 6.2 – 9.6 µm long, with a length to diameter ratio of 1:1 to 3.2:1. The intercalary plurilocular structures were uniseriate and borne near the apex of the filaments capped by two to three enlarged sterile terminal cells (Fig. 4.11e). The plurilocular bodies measured 11.6 – 17.5 µm in length and 4.5 – 5.0 µm wide. As for the second morphotype, the thallus comprised a two-partitioned form of erect filaments, in which the upper half are laterally free while the lower half are laterally cohesive, arising curvedly from a two-celled (13.0 – 25.4 µm) thick basal layer (Fig. 4.11f). The cells of the basal plate were 10.9 – 28.2 µm wide, 4.6 – 10.1 µm long, or two to four times wider than long. The erect filaments were longer, reaching up to 190 µm for those bearing plurilocular bodies and up to 232 µm long for those bearing unilocular

structures. Erect filaments bearing plurilocular bodies consisted of eight to 12 cells (excluding sterile terminal cells and plurilocular structures). These cells were 4.3 - 13.1 µm in diameter, 4.7 - 13.3 µm in length, with a length to diameter ratio of 0.5:1 to 2.7:1. Plurilocular structures were initially uniseriate, later biseriate, measuring 18.3 - 62.1 µm long and 6.9 - 10.2 µm in diameter, inserted near the apex of the erect filaments, and capped by a single dome-shaped sterile terminal cell measuring 12.5 - 17.1 µm long and 6.3 - 10.0 µm wide (Fig. 4.11g). Erect filaments bearing unilocular structures comprised eight to 16 cells. Cells at the upper half of the filaments were more elongated, measuring 4.0 - 8.8 µm wide, 4.9 - 14.1 µm long or 0.8 to 3.2 times longer than wide. Meanwhile, cells at the lower half of the filaments were 5.5 - 12.7 µm wide, 5.7 - 11.7 µm long or 0.7 to 1.9 times as long as wide. Unilocular reproductive structures were 7.3 - 54.5 µm in diameter and 15.9 - 99.6 µm in length, singly borne on one to two-celled stalks, sometimes sessile, terminal on the lower half erect filaments, lateral and basal to the upper portion erect filaments, i.e. inserted in the middle of the erect filaments (Fig. 4.11h). Hair tufts were infrequently observed emerging from a depression in the basal layer.

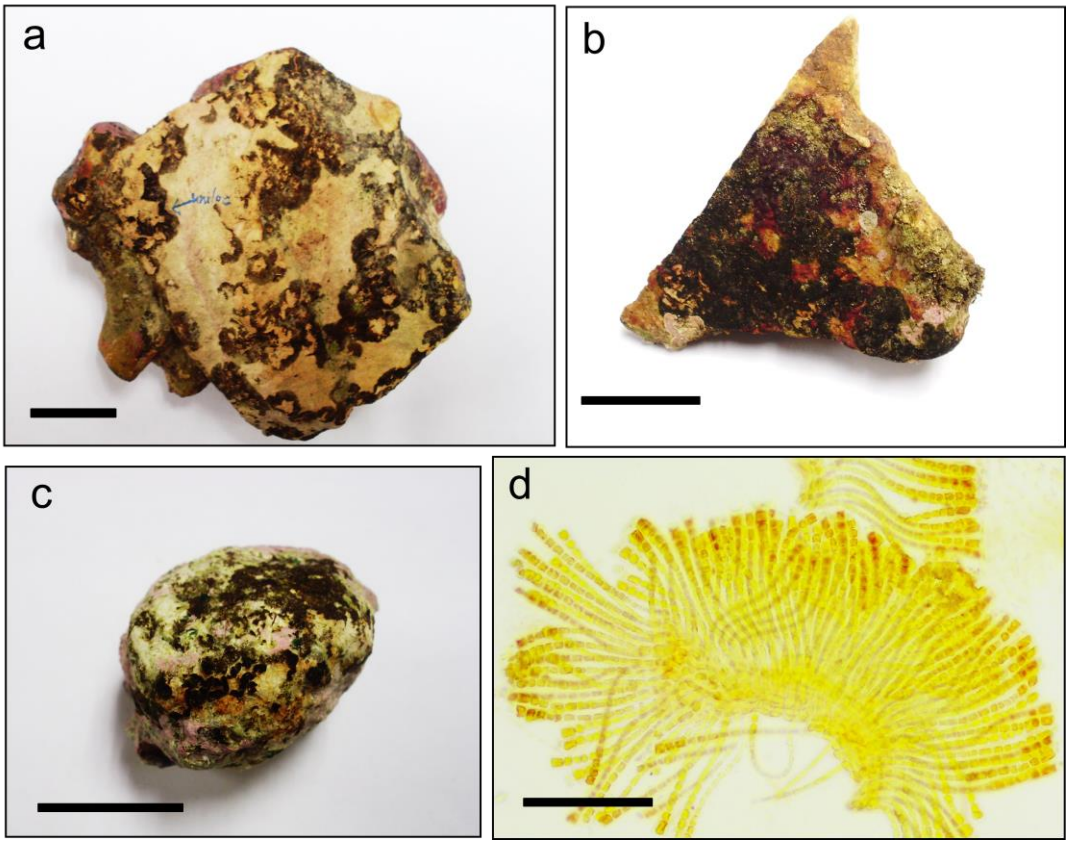

Figure 4.11 *Mesospora negrosensis* (a – c) Various morphologies of thalli on substratum [Voucher no. = PSM 12183, PSM 12326 and PSM 12251, respectively] Scale bars = 2 cm. (d) Morphotype 1: Vegetative filaments, laterally free throughout except at the basal plate [Voucher no. = PSM 12183] Scale bar = 100 µm.

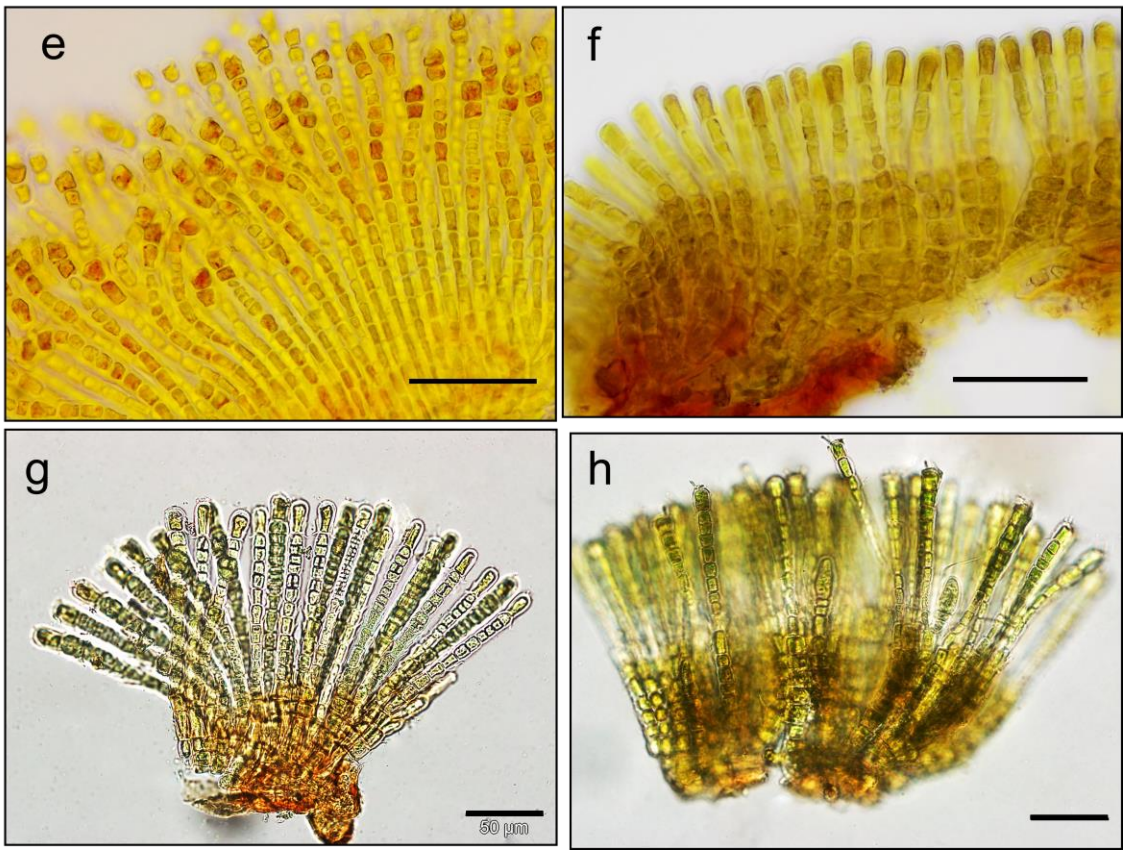

Figure 4.11 (continued) *Mesospora negrosensis* (e) Morphotype 1: Uniseriate plurilocular reproductive structures borne near the apex of the erect filaments and capped by two to three sterile terminal cells [Voucher no. = PSM 12183] (f) Morphotype 2: Vegetative filaments, laterally free at the distal end and more tightly adjoined near the basal end [Voucher no. = PSM 12171] (g) Morphotype 2: Plurilocular structures borne near the apex of the erect filaments, initially uniseriate later biseriate and capped by a single sterile terminal cell [Voucher no. = PSM 12251] (h) Morphotype 2: Unilocular reproductive structures inserted terminally on two stalk cells, lateral to and in the middle of the erect filaments [Voucher no. = PSM 12324_117A] Scale bars = 50 µm.

4.3.1.3 *Mesospora elongata* Poong, Lim & Phang, Fig. 4.12

Thallus greenish brown to dark brown, thin and epilithic, initially circular in outline, later coalescent with surrounding thalli, entire lower surface tightly adherent to the substratum without rhizoids (Figs. 4.12a – b). Thallus composed of thin (generally two) layers of horizontal basal cells wider than high and erect filaments of up to 30 cells long were laterally free throughout (Fig. 4.12c). Fertile parts of the thallus were particularly gelatinous upon contact with water. Cells near the basal end of the erect filaments were more cylindrical measuring 6.3 - 15.9 (-23.7) μm in length and 2.5 - 6.0 (-9.2) μm in width; cells at the distal end were somewhat isodiametric measuring 5.8 - 10.7 (-17.1) μm in length and 3.4 - 7.9 (-13.2) μm in width. The plurilocular reproductive structures were biseriate and capped by two to four sterile terminal cells near the apex of the erect filaments (Fig. 4.12d). Plurilocular bodies were (13.8) 22.1 - 49.9 μm in length, (4.8) 8.1 - 10.9 μm in width, and the locules were sometimes uniseriate. The erect filaments bearing unilocular structures are distinct from those bearing plurilocular bodies, having a smaller number of cells (11 - 16 cells) per erect filament and the cells terminating the erect filaments tended to be elliptical (compare Fig. 4.12e to Fig. 4.12c). Unilocular reproductive structures were rare (observed in only one specimen), terminally inserted on up to ten stalk cells, sometimes sessile, lateral and basal or middle/sub-superficial in relation to the parent erect filament and surrounding filaments (Fig. 4.12f). Occasionally, it is unclear whether the stalk cells have arisen directly from the basal plate as the parent erect filament may be inadvertently hidden from view. Unilocular structures were 25.9 - 76.7 μm in length and 11.5 - 26.9 μm in diameter. Hair tufts were occasionally observed. This species is characterised by its large number of cells per erect filament and unilocular structures borne on long stalks.

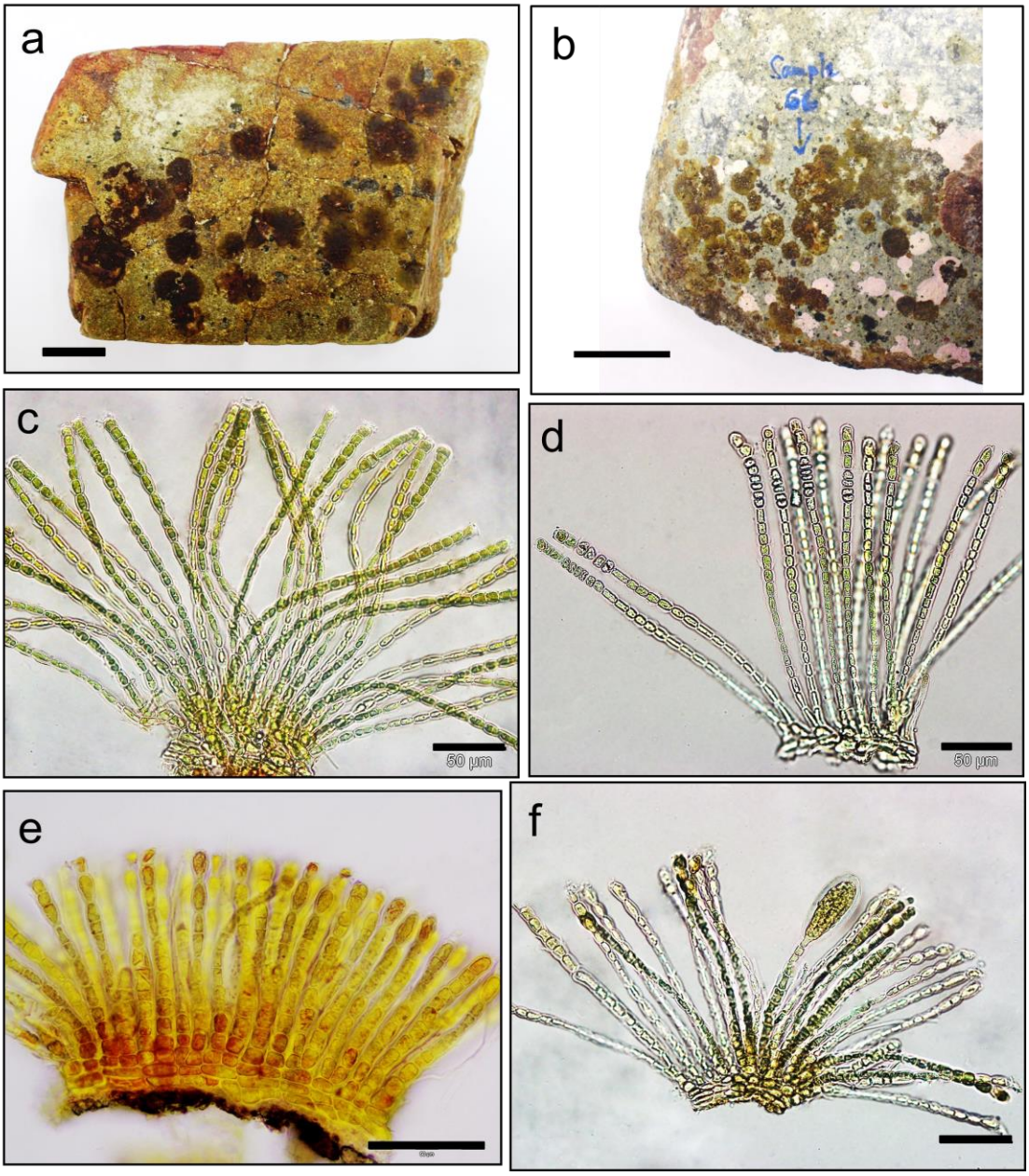

Figure 4.12 *Mesospora elongata* (a - b) Thalli on substratum [Voucher no. = PSM 12234 and PSM 12214, respectively] Scale bars = 2 cm. (c) Vegetative filaments before development of plurilocular bodies [Voucher no. = PSM 12234] (d) Plurilocular bodies borne near the apex of erect filaments terminated by two to three sterile cells [Voucher no. = PSM 12214] (e) Conspicuously shorter vegetative filaments before development of unilocular reproductive structures [Voucher no. = PSM 12221] (f) A unilocular reproductive structure terminally inserted on a long stalk and lateral to the surrounding erect filaments [Voucher no. = PSM 12221] Scale bars = 50 µm.

4.3.1.4 *Mesospora* sp. C, Fig. 4.13

This species was first reported by Lim et al. (2007) from Tanjong Gemoh, Malaysia. Thallus dark brown to black, thin and epilithic, initially circular in outline later becoming indefinite, frequently confluent with thalli of other crustose brown algal species, particularly *M. schmidtii* (Figs. 4.13a – b). Thallus, especially fertile ones are gelatinous upon contact with water. The entire thallus was adherent to the substratum without rhizoids. Thallus consisting of two to three layers of prostrate filaments approximately 7 - 15 µm thick from which erect filaments arise (Fig. 4.13c). Cells of the basal layer measured 2.7 – 4.5 µm in length and 2.4 to 4.2 times as broad as long whereas cells of the erect filaments were 3.7 – 9.5 µm in diameter and 5.8 – 19.8 µm long, with a length to diameter ratio of 1:1 to 2.5:1. The erect filaments were composed of eight to 17 cells, up to approximately 230 µm long, somewhat closely packed and not as readily separated as in *M. schmidtii* and *M. elongata*. The plurilocular reproductive structures of this species are distinctively short compared to other species of *Mesospora*, at first uniseriate and later biseriate (Fig. 4.13d). In most cases, these structures developed from the second and third cells from the apex of the erect filament, with each of the cells divided into four loculi. The plurilocular bodies measured 5.7 – 10.8 µm in diameter and 13.3 – 26.9 µm in length, and capped by a dome-shaped sterile terminal cell. Unilocular reproductive structures were not observed. Hair tufts were occasionally present.

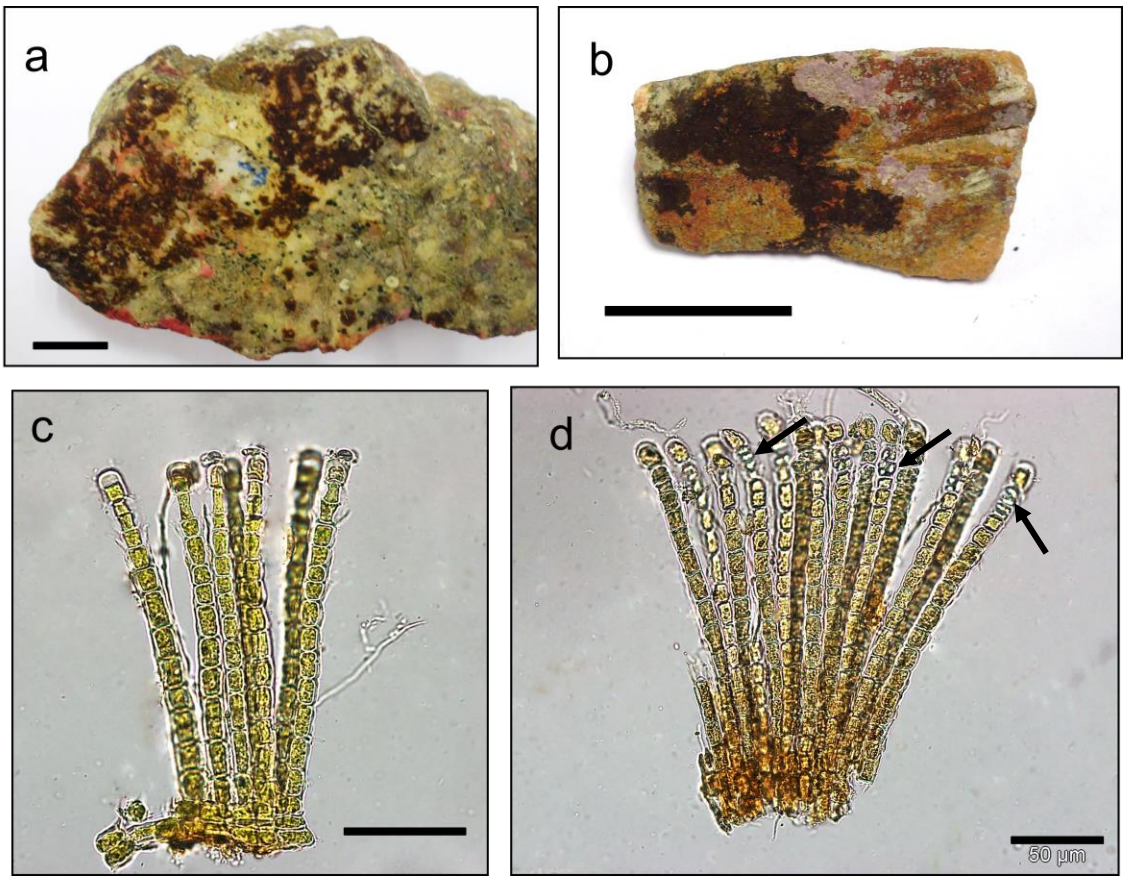

Figure 4.13 *Mesospora* sp. C (a-b) Thalli on substratum [Voucher no. = PSM 12205 and PSM 12338, respectively] Scale bars = 2 cm. (c) Vegetative filaments arising from a distromatic basal plate, prior to the development of plurilocular bodies [Voucher no. = PSM 12346_140E] (d) Short plurilocular reproductive structures (indicated by arrows) inserted near the apex of erect filaments and capped by a single sterile terminal cell [Voucher no. = PSM 12205] Scale bars = 50 µm.

4.3.1.5 *Mesospora* sp. D, Fig. 4.14

This species was first reported by Lim et al. (2007) from Nakura, Japan. Thallus thin, epilithic, forming circular to irregular crusts, greenish to blackish brown, gelatinous upon contact with water (Figs. 4.14a – b). Thallus is firmly adherent to substratum without rhizoids and often coalescent with surrounding thalli. The thallus is composed of erect filaments arising from a basal plate which is two to three cells thick. Cells of the basal plate measured 6.4 – 23.4 µm wide, 3.9 – 13.2 µm long and the length to diameter ratio was 0.3:1 to 0.9:1. Cells of the erect filament numbered eight to 17, measuring 3.8 – 9.9 µm in diameter and 5.9 – 14.0 µm long with a length to diameter ratio of 0.7:1 to 2.5:1. In the young thallus, the upper two third portion of the erect filaments was laterally free while the basal one third portion (~ three cell layers) was somewhat more laterally cohesive, thus giving a false impression of a thick basal layer (Fig. 4.14c). In this context, it is similar to the second morphotype of *M. negrosensis* but with a larger number of sterile terminal cells. The mature erect filaments were laterally free throughout. Structures believed to be developing (and uniseriate?) plurilocular bodies terminated by two to three sterile cells near the apex of the erect filaments were observed in the samples from Lombok Island (Fig. 4.14d) and Nakura, Japan. Unilocular reproductive structures were not observed. Hairs were infrequently seen.

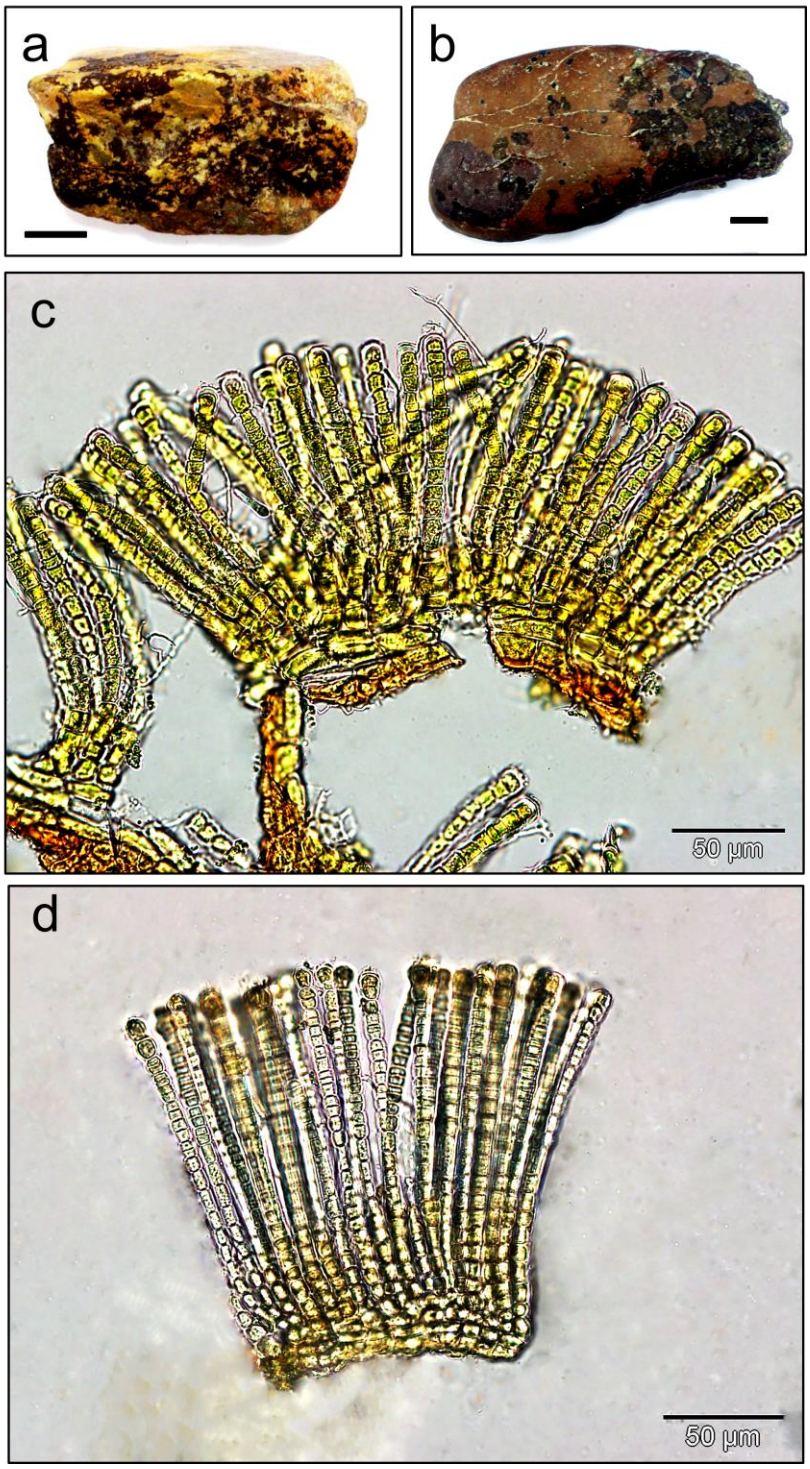

Figure 4.14 *Mesospora* sp. D (a - b) Thalli on substratum [Voucher no. = PSM 12175 and PSM 12188, respectively]. Scale bars = 2 cm. (c) Vegetative filaments at a younger stage [Voucher no. = PSM 12346_140F] (d) Erect filaments on the verge of developing plurilocular reproductive structures near the apex of the filaments [Voucher no. = PSM 12220]. Scale bars = 50 µm.

4.3.1.6 *Mesospora* sp. 1, Fig. 4.15

Thallus thin and epilithic, greenish brown to almost black, sometimes with an eroded centre (Figs. 4.15 a - c). Thallus (especially fertile parts) was gelatinous upon contact with water, closely attached to substratum without rhizoids, and the outline was initially circular later beame irregular and often confluent with surrounding thalli. Erect filaments arised from a basal disc of two to three horizontal cell layers, approximately 16 to 26 µm thick, with cells measuring 7.3 – 25.1 µm wide and 3.4 – 10.9 µm long. The young thallus of this species is very similar to the young thallus of *Mesospora* sp. D (Fig. 4.15d). The small difference between the two is that instead of the ratio being distal two-third laterally free and basal one-third laterally cohesive, the ratio here is half to half. The lower half erect filaments of its thallus were rather closely packed, requiring more pressure to be separated as compared to *M. schmidtii* and *M. elongata*. The erect filaments were at least 68 µm long and can reach up to 220 µm long. Number of cells of the erect filaments ranged from six to 18; the cell dimensions were 3.2 – 12.2 µm broad and 5.7 - 20.4 µm long, with the length to diameter ratio of 0.6:1 to 3.5:1. Plurilocular bodies were at first uniseriate, later biseriate and capped by two to three prominent and (usually) highly pigmented sterile terminal cells (Fig. 4.15e). The plurilocular bodies measured 7.7 – 12.8 µm in diameter and 27.5 – 59.2 µm in length. Unilocular reproductive structures were terminally borne on one to two stalk cells lateral to and in the middle of the erect filaments (Fig. 4.15f). The unilocular structures were 18.8 – 37.4 µm in diameter and 37.1 – 70.7 µm long, with a length to diameter ratio of 1.4:1 to 2.5:1. Hair tufts emerging from depressions in the basal layer were occasionally observed.

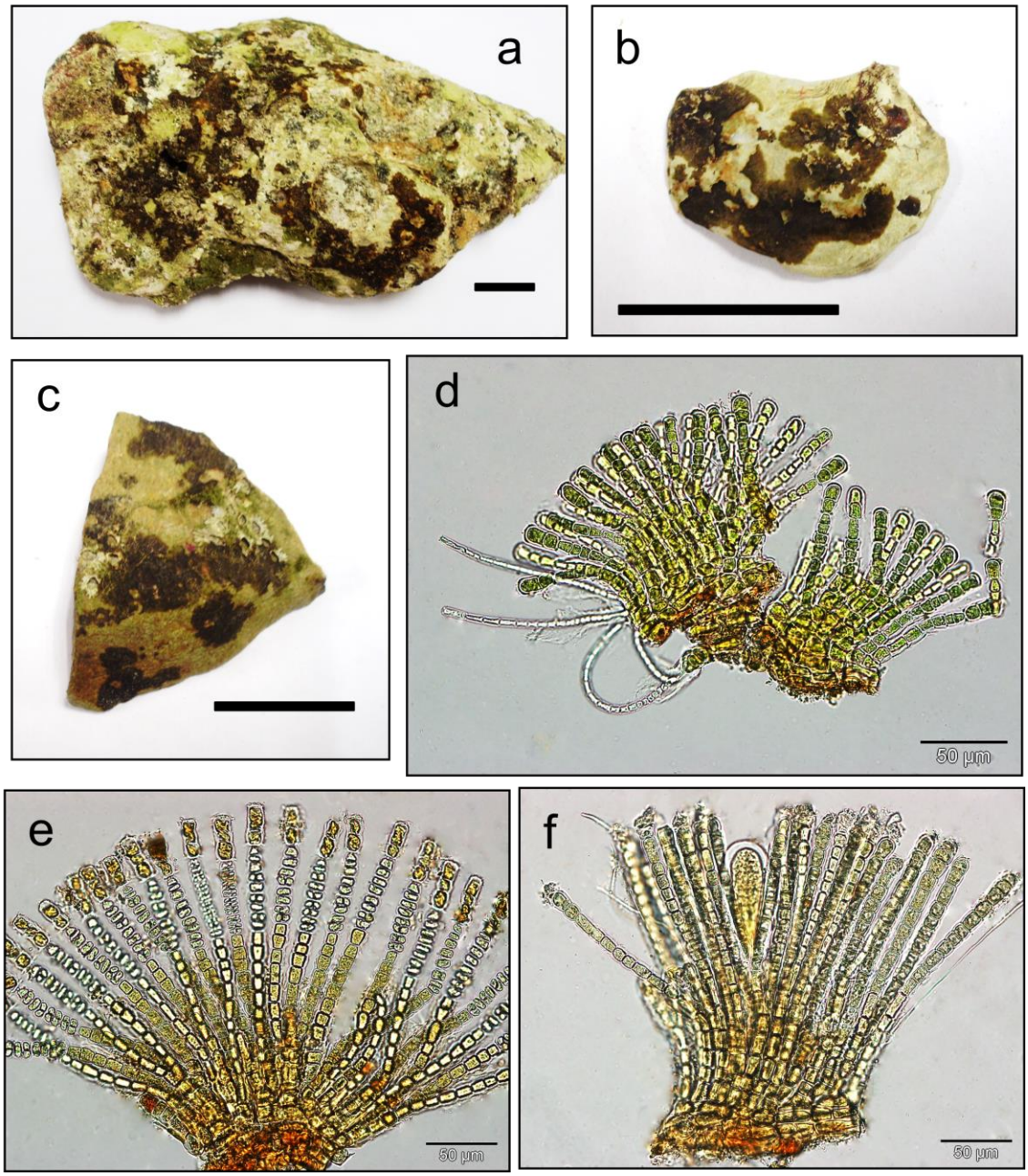

Figure 4.15 *Mesospora* sp. 1 (a - c) Various morphologies of thalli on substratum [Voucher no. = PSM 12212, PSM12321 and PSM 12320, respectively] Scale bars = 2 cm. (d) Young, vegetative filaments with hair tufts emerging from a depression in the basal plate [Voucher no. = PSM 12346_140F] (e) Plurilocular reproductive structures borne near the apex of the erect filaments, capped by two to three sterile terminal cells [Voucher no. = PSM 12212] (f) A unilocular reproductive structure inserted among the erect filaments [Voucher no. = PSM 12232] Scale bars = 50 µm.

4.3.1.7 *Mesospora* sp. 2, Fig. 4.16

Thallus thin, epilithic, light to dark brown, sometimes with an eroded centre, attached firmly to the substratum without the presence of rhizoids (Figs. 4.16a – b). The thallus (especially fertile parts) was mucilaginous upon contact with water. This species was frequently found on the same substratum as *Diplura* sp. 2 and both can be distinguished based on the colour of their thalli. The outline of its thallus was initially circular, later tended to be indefinite and sometimes the thalli overlapped on one another. This species is unique in its thallus construction in which a three-layered structure can be clearly seen in thalli bearing plurilocular bodies (Fig. 4.16c), which is somewhat reminiscent of the delineation between cortical and medullary layers in *Neoralfsia expansa*. However, unlike *N. expansa*, the thallus does not have a bilaterally symmetrical structure. The thallus was composed of prostrate filaments from which upwardly curving and laterally cohesive filaments arise. The basal plate was thin, approximately 10 - 12 µm in thickness, with cells measuring three to four times as wide as long. Differentiation of the erect filaments may take place in two ways, depending on whether it develops into a thallus bearing plurilocular or unilocular reproductive structures. If development of plurilocular reproductive structures were to take place, the uppermost portion of the erect filaments differentiates into plurilocular reproductive structures capped by a sterile terminal cell (Fig. 4.16d), and this corresponds to the first layer. The plurilocular bodies were at first uniseriate and later biseriate, measuring 14.9 – 58.1 µm long and 4.8 – 12.0 µm wide. The central layer comprised cells directly below the plurilocular reproductive structures and the cells were 4.7 – 8.4 µm wide and 5.3 – 13.6 µm long (the length to diameter ratio was 0.8:1 to 2.3:1) whereas cells of the bottom layer are more elongated measuring 6.1 – 11.7 µm in diameter and 10.9 – 31.2 µm in length (the length to diameter ratio was 1.3:1 to 4.9:1), and sometimes branched (Fig. 4.16e). The delineation

of the second and third layers may not be apparent, especially when the bottom layer and basal plate were disrupted during the squash preparation process (Fig. 4.16f). In contrast, in preparation for the development of unilocular reproductive structures, cells at the upper half of the erect filaments become more elongated measuring 3.4 – 8.3 µm broad and 5.1 – 15.5 µm long (length to diameter ratio of 0.8:1 to 3.2:1) and resembled a club-shaped paraphysis-like structure (Fig. 4.16g). Meanwhile cells at the lower half layer were 4.2 – 9.4 µm in diameter and 5.7 – 23.7 µm long, with the length to diameter ratio of 0.8:1 to 2.7:1. The three-layered thallus structure is not clearly seen in thallus bearing unilocular structures and the lower half portion of the erect filaments were often detached during the squashing process. Unilocular reproductive structures were terminally inserted on one to three stalk cells, terminal to the lower half erect filaments and basal-lateral to the upper half erect filaments (Figs. 4.16h - i). In other words, they were inserted in the middle of the erect filaments. The unilocular structures were 9.1 – 33.8 µm in diameter and 29.9 – 88.3 µm in length, with the length to diameter ratio of 2.1:1 to 4.1:1. Hairs were infrequently observed.

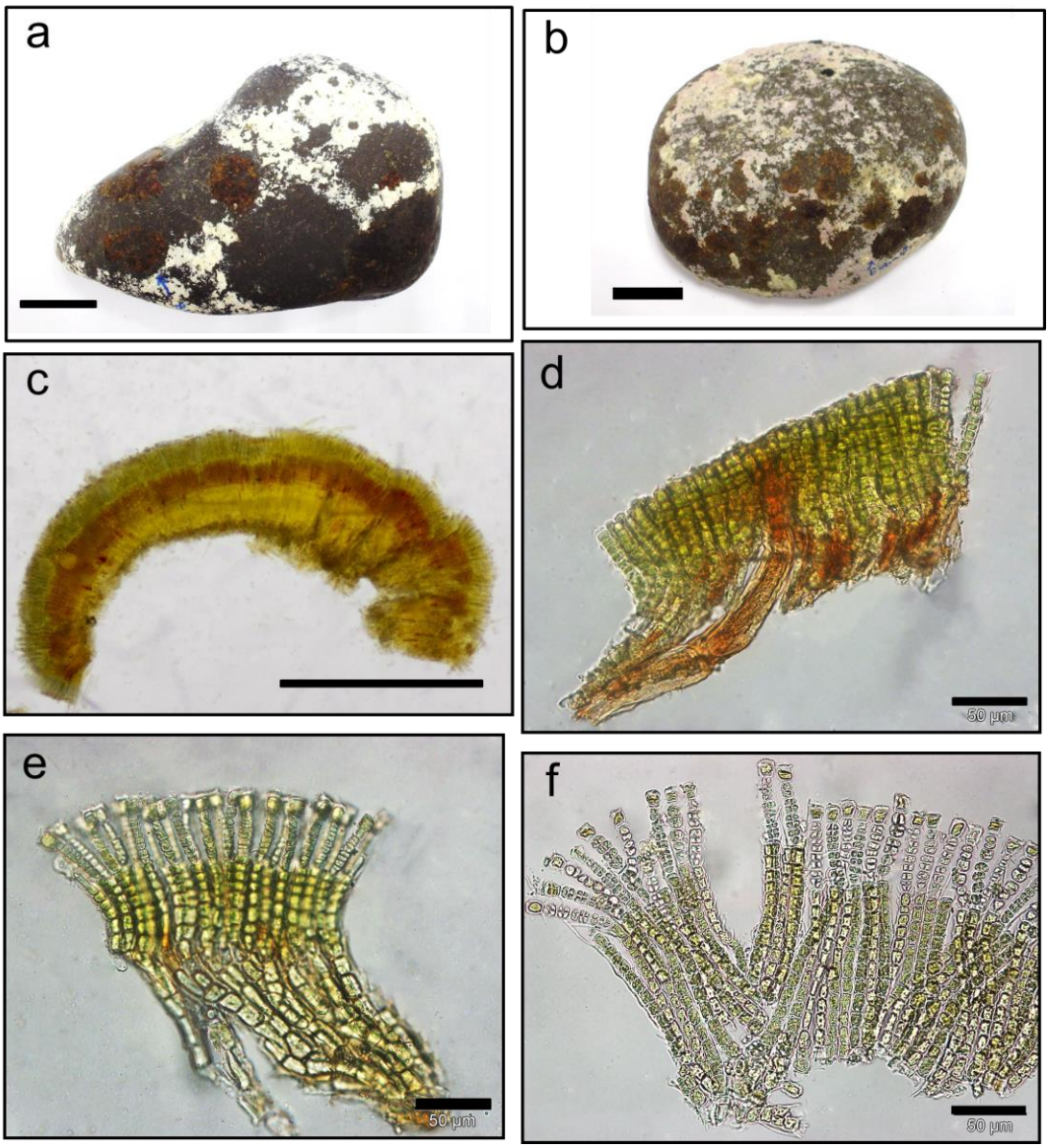

Figure 4.16 *Mesospora* sp. 2 (a – b) Thalli on substratum [Voucher no. = PSM 12241 and PSM 12211] Scale bars = 2 cm. (c) Thallus with three distinct layers [Voucher no. = PSM 12239] Scale bar = 500 µm. (d) Development of plurilocular bodies from the erect filaments [Voucher no. = PSM 12242_83] (e) Largely uniseriate plurilocular reproductive structures capped by a sterile terminal cell, showing distinct layering [Voucher no. = PSM 12241_79] (f) Biseriate plurilocular reproductive structures capped by a sterile terminal cell, with the basal layer damaged during squashing of the thallus [Voucher no. = PSM 12239] Scale bars = 50 µm.

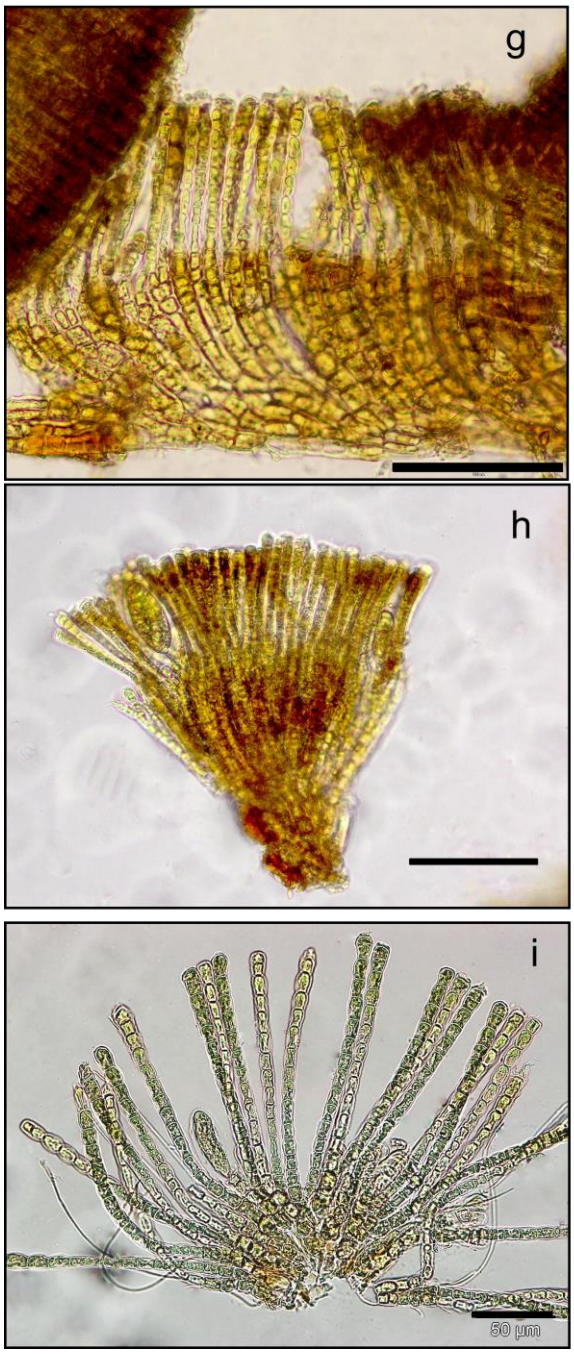

Figure 4.16 (continued) *Mesospora* sp. 2 (g) Vegetative filaments before development of unilocular reproductive structures [Voucher number = PSM 12241_81] (h) Unilocular structures terminally inserted on stalk cells, lateral and in the middle of the erect filaments [Voucher number = PSM 12240_76] Scale bars = 100 µm.(i) Unilocular structures terminally inserted on stalk cells, lateral and in the middle of the erect filaments of which the basal plate was damaged during squashing of the thallus [Voucher number = PSM 12239]. Scale bar = 50 µm.

4.3.2 "*Mesospora*" sp. 3, Fig. 4.17

This taxon is provisionally named "*Mesospora*" due to its thin thallus resembling that of *Mesospora*. Thallus greenish brown to black, indefinite in outline, often coalescent with other thalli, fertile parts were slightly mucilaginous upon contact with water, and the whole thallus attached firmly to the substratum without anchorage by rhizoids (Figs. 4.17a - b). The sterile thallus was made up of a two to four-celled tall basal plate from which tightly bound erect filaments arise. Cells of the basal layer were 7.9 – 16.5 µm wide and 2.3 – 6.5 µm long. The erect filaments comprised approximately ten to 14 cells, 53.5 – 107.9 µm in length and occasionally branched. Cells of the erect filaments were 3.5 – 8.1 µm broad and 3.0 – 7.3 µm long, the length to diameter ratio was 0.5:1 to 1.6:1. Prior to the formation of unilocular reproductive structures, multicellular and club-shaped paraphyses were formed above the erect filaments. The unilocular structures measured 15.8 – 39.7 µm in length and 6.8 – 24.3 µm in width, the length to diameter ratio was 1.2:1 to 3:1. They were terminally borne on one to two (generally one) celled stalks, terminal on the erect filaments and lateral-basal to the paraphyses (Figs. 4.17c and e). The paraphyses accompanying the unilocular structures were thick walled and consisted of four to 11 cells. They were 41.7 – 85.2 µm long and 2.0 – 7.5 µm broad. Plurilocular bodies were mostly uniseriate, capped by a sterile terminal cell (highly pigmented at times) and inserted above the erect filaments (Figs. 4.17d and f). The plurilocular structures measured 3.2 – 7.5 µm broad and 10.5 – 28.0 µm long. Hair tufts were occasionally observed. This potentially new genus is distinguished from *Mesospora* on the basis of its smaller cell dimension (considerably shorter and much more isodiametric, to be accurate) and the unilocular reproductive structures were accompanied by paraphyses.

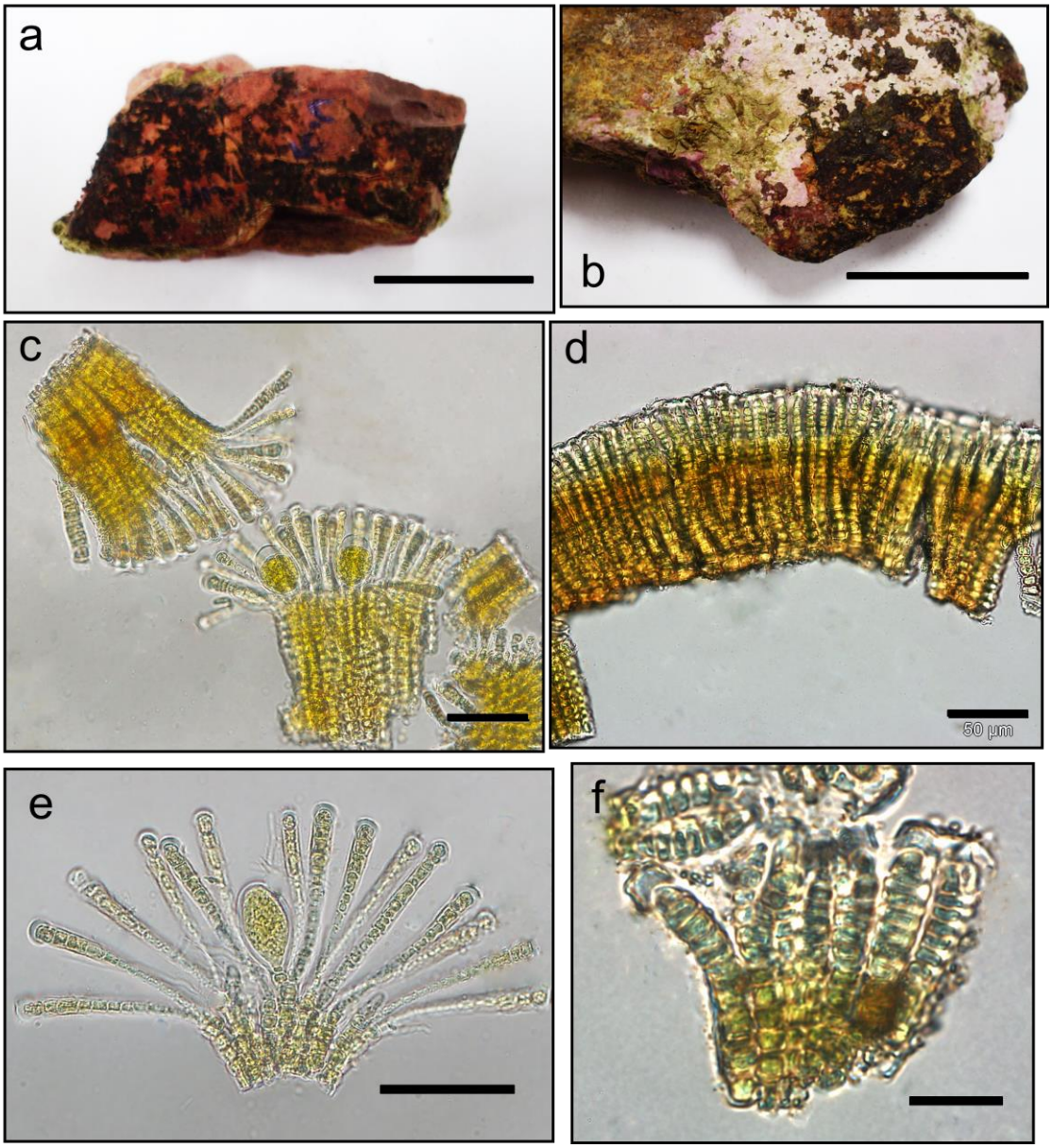

Figure 4.17 "*Mesospora*" sp. 3 (a – b) Thalli on substratum [Voucher no. = PSM 12189 and PSM 123352, respectively] Scale bars = 2 cm. (c) Unilocular reproductive structures terminally inserted on the erect filaments, lateral to the paraphyses [Voucher no. = PSM 12775] (d) Uniseriate plurilocular structures terminally borne on the erect filaments [Voucher no. = PSM 12352] (e) A close-up of a unilocular structure inserted on stalk cells [Voucher no. = PSM 12775] Scale bars = 50 µm. (f) A close-up of the plurilocular bodies, seemingly capped by a sterile terminal cell [Voucher no. = PSM 12352] Scale bar = 20 µm.

4.3.3 *Diplura* spp.

Members of *Diplura* are noted for their relatively thin thallus. Their vegetative filaments were somewhat tightly adherent, not so much as in *Neoralfsia expansa* but more cohesive than *Mesospora*. Plurilocular reproductive structures were reported in every species recorded while unilocular reproductive structures were present only in certain species. Several chloroplasts were present in each cell. Currently, the four genetic species of *Diplura* identified in this study were morpho-anatomically indistinguishable.

4.3.3.1 *Diplura* sp. 1, Fig. 4.18

Thallus epilithic, smooth, dark brown to black, very thin, somewhat circular in outline when young, later often confluent with other thalli (Figs. 4.18a - c). Thallus was wholly adherent to the substratum without rhizoids, rather difficult to be removed from the substratum due to its thinness. The fertile thallus was slightly gelatinous upon contact with water. Thallus consisted of a distromatic plate giving rise to vertical, little branched and firmly adjoined vegetative filaments. Cells of the basal layer were 10.0 – 14.2 µm broad, 2.6 – 5.7 µm long, the length to diameter ratio was 0.2:1 to 0.6:1. Cells of the erect filaments numbered five to seven (excluding the plurilocular bodies and sterile terminal cells), mostly 4.2 – 9.5 µm in diameter and 0.4 to 1.7 times as long as wide. Plurilocular reproductive structures were initially uniseriate later biseriate, 15.5 – 28.4 µm in length and 6.8 – 11.6 µm in diameter and capped by a sterile terminal cell (Fig. 4.18d). Unilocular reproductive structures and hairs have not been observed for this species.

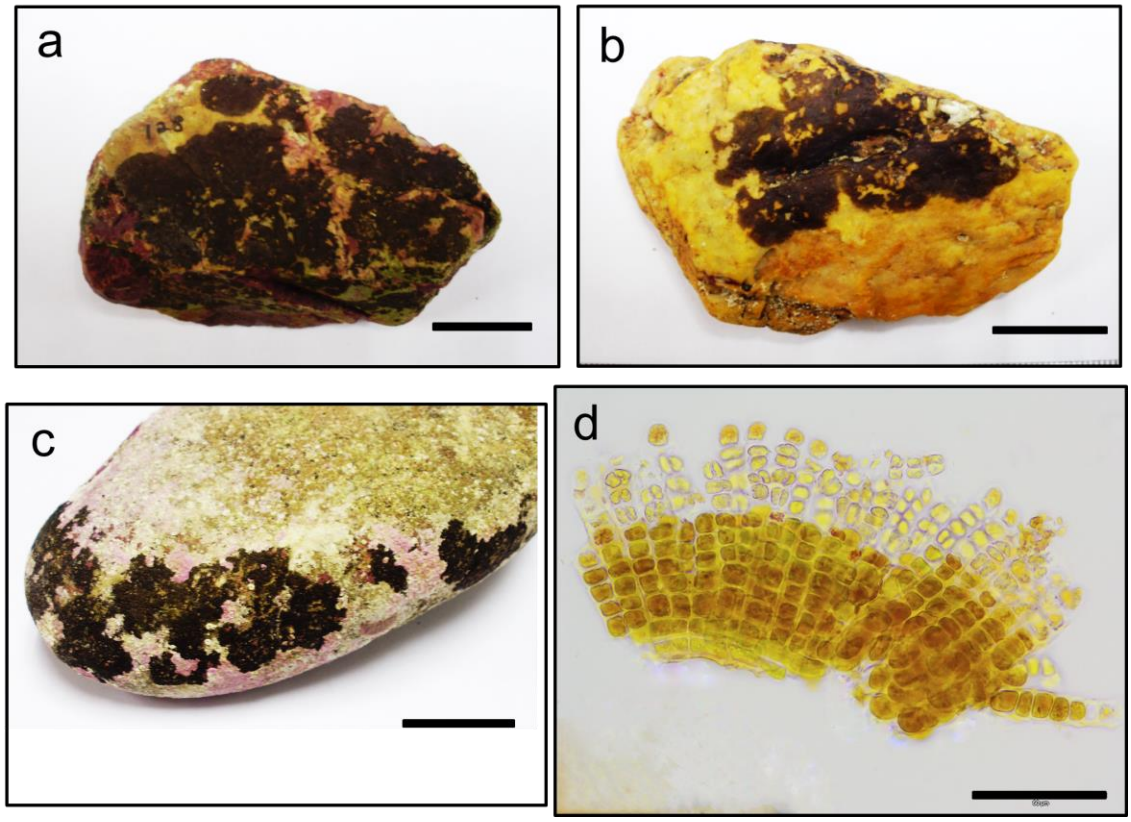

Figure 4.18 *Diplura* sp. 1 (a – c) Various morphologies of thalli on substratum [Voucher no. = PSM 12334, PSM 12373 and PSM 12359, respectively] Scale bars = 2 cm. (d) Plurilocular reproductive structures capped by a sterile cell [Voucher no. = PSM 12208] Scale bar = 50 µm.

4.3.3.2 *Diplura* sp. 2, Fig. 4.19

Thallus dark brown to black, outline circular to irregular, thicker than the other three *Diplura* species, and firmly attached to the substratum by its under surface without rhizoids (Figs. 4.19a – b). Surface of thallus smooth and suede-like, fertile parts were slightly gelatinous upon contact with water. Vegetative thallus composed of a distromatic basal layer from which erect filaments arise. Cells of the basal layers measured 3.2 – 6.5 µm in height and 2.0 – 4.5 times as wide as high. The erect filaments were composed of nine to 14 cells (excluding the plurilocular bodies and sterile terminal cells), laterally cohesive throughout, the cells were 5.6 – 10.1 µm broad, 2.6 – 8.0 µm long and the length to diameter ratio was 0.3:1 to 1.2:1. Plurilocular reproductive structures are biseriate, capped by a sterile terminal cell and measured 17.2 - 34.6 µm long and 7.1 – 10.6 µm wide (Fig. 4.19c). Unilocular reproductive structures were terminally inserted on a single-celled stalk, lateral and basal to the multicellular paraphyses and terminal on the vegetative filaments (Fig. 4.19d). The unilocular structures were 6.5 – 15.8 µm wide and 17.2 – 46.1 µm long. The paraphyses were 44.9 - 67.5 µm long and 4.2 – 9.1 µm wide, consisting of four to five cells. Hairs were not observed. This species was frequently found in close proximity with the crusts of *Mesospora* sp. 2.

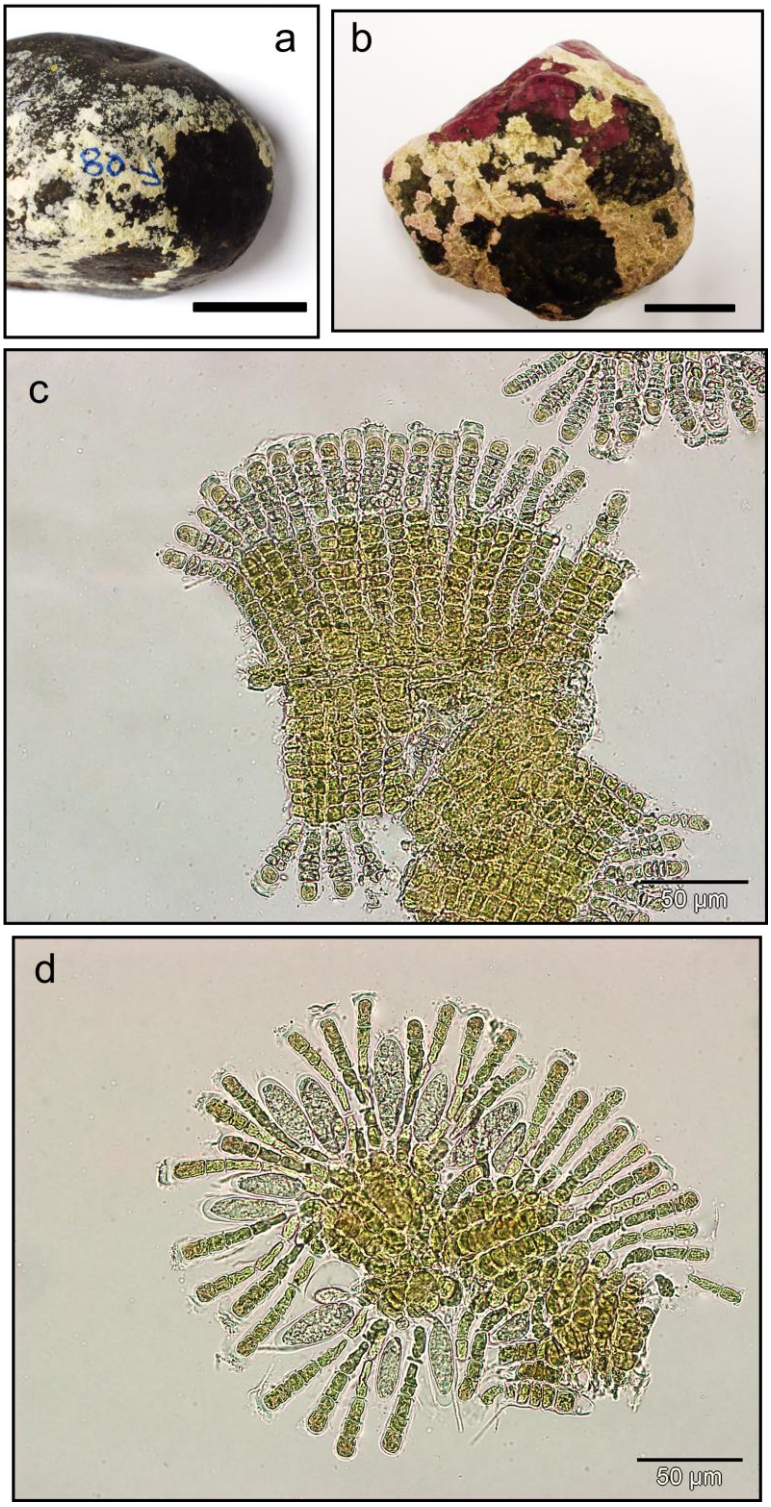

Figure 4.19 *Diplura* sp. 2 (a – b) Thalli on substratum [Voucher no. = PSM 12241_80 and PSM 12314] Scale bars = 2 cm. (c) Plurilocular reproductive structures capped by a sterile cell [Voucher no. = PSM 12236_47A] (d) Unilocular reproductive structures terminally inserted on stalk cells and lateral-basal to the paraphyses [Voucher no. = PSM 12237] Scale bars = 50 µm.

4.3.3.3 *Diplura* sp. 3, Fig. 4.20

Thallus very thin, greenish brown to black, initially circular in outline, later tended to be indefinite and coalescent with surrounding thalli, closely adherent to the substratum by whole under surface, rhizoids not observed (Figs. 4.20a - b). Fertile portions of the thallus were slightly gelatinous upon contact with water. Thallus consisted of a distromatic base giving rise to simple, straight and rather closely packed erect filaments. Cells of the erect filament numbered seven to ten (excluding the plurilocular bodies and sterile terminal cells), measuring 4.9 – 10.9 µm broad and 3.5 – 15.4 µm long, the length to diameter ratio was 0.4:1 to 1.8:1. Unilocular reproductive structures were rare (found in only one specimen), terminally inserted on one to two stalk cells, lateral-basal to the multicellular (three to six celled) paraphyses and terminal to the erect filaments (Fig. 4.20c). The unilocular structures were oval, measuring 6.5 – 12.2 µm wide and 19.1 – 27.8 µm long. More than one unilocular reproductive structure may be inserted on a single erect filament. The paraphyses were 38.6 – 57.7 µm long and 4.9 – 7.0 µm broad. Plurilocular bodies were subterminally inserted on the erect filaments, initially uniseriate, later biseriate and capped by a sterile terminal cell (Figs. 4.20d - f). The plurilocular bodies were 6.1 – 11.6 µm wide and 17.9 – 40.1 µm long. One or two reproductive filaments (when there is branching of vegetative filaments), each terminated by a sterile cell, were inserted on a vegetative filament. Hairs were not observed.

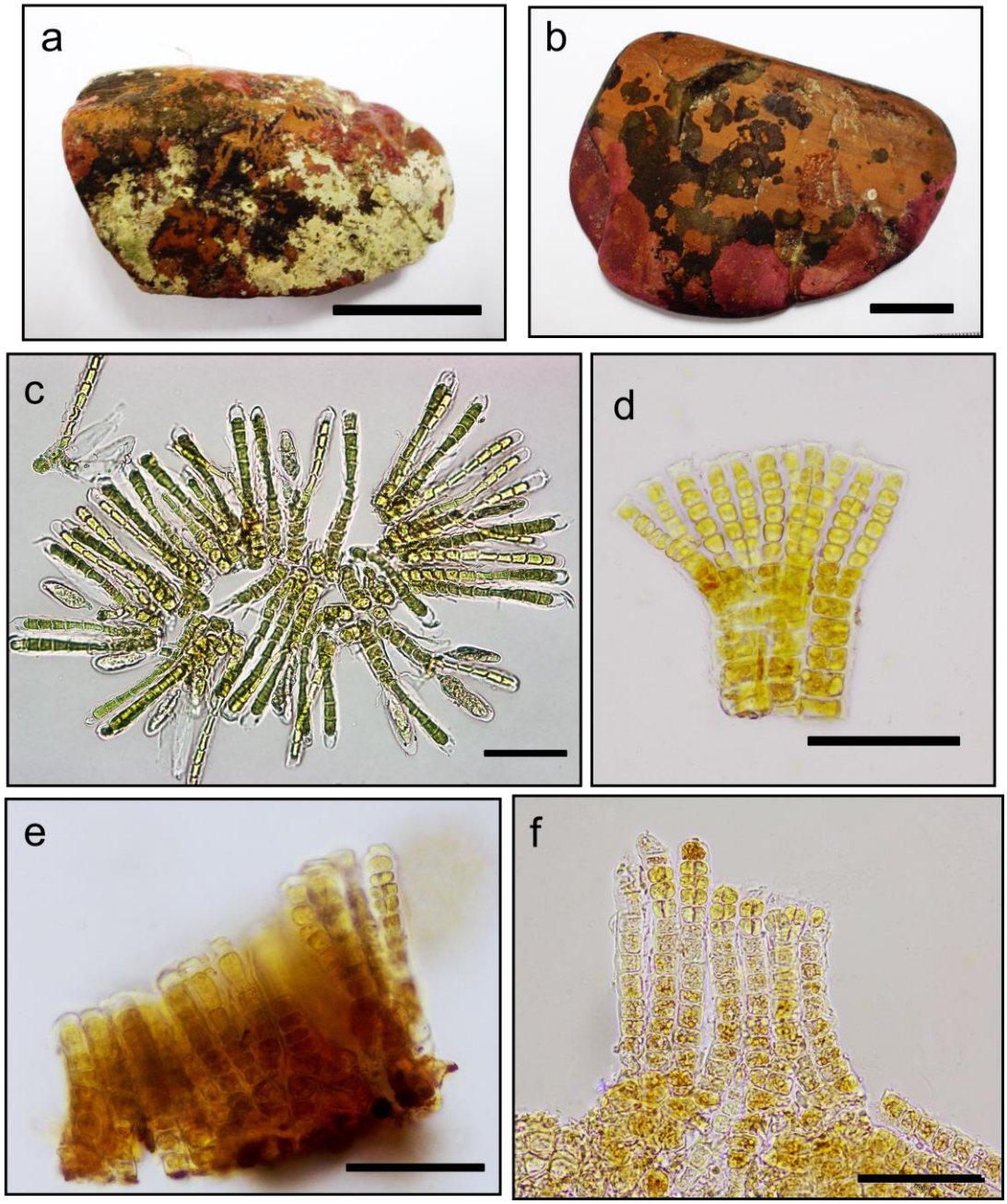

Figure 4.20 *Diplura* sp. 3 (a – b) Thalli on substratum [Voucher no. = PSM 12172 and PSM 12193, respectively] Scale bars = 2 cm. (c) Unilocular structures inserted on a stalk cell, lateral-basal to paraphyses and terminal on the erect filaments [Voucher no. = PSM 12172] (d) Developing plurilocular bodies, a mixture of one or two reproductive filaments per vegetative filament, each terminated by a sterile cell [Voucher no. = PSM 12216] (e) Plurilocular bodies developing via differentiation of the terminal cells [Voucher no. = PSM 12209] (f) Mature biseriate plurilocular structures terminated by a highly pigmented sterile cell [Voucher no. = PSM 12224_48B] Scale bars = 50 µm.

4.3.3.4 *Diplura* sp. 4, Fig. 4.21

Thallus dark brown to black, epilithic, very thin, irregular in outline, often coalescent with surrounding thalli, whole thallus closely adherent to substratum without anchorage by rhizoids (Figs. 4.21a - b). Fertile parts of the thallus were slightly gelatinous upon contact with water. Thallus consisted of a thin layer (probably two-celled thick) of prostrate filaments from which simple, sparsely branched erect filaments arise. The erect filaments were rather tightly adjoined, approximately 100 – 120 µm long, comprising eight to 13 cells (excluding the plurilocular bodies and sterile terminal cells) measuring 4.7 – 9.5 µm wide and 4.3 – 9.3 µm long, the length to diameter ratio 0.5:1 to 1.5:1. Plurilocular reproductive structures were inserted near the apex of the erect filaments, initially uniseriate, later biseriate, and capped by a sterile terminal cell (Fig. 4.21c). The plurilocular bodies were 7.54 – 9.8 µm in diameter and 9.6 – 24.4 µm in length. One or two reproductive filaments, each terminated by a sterile cell, were inserted on a vegetative filament. Unilocular reproductive structures were either sessile, or terminally inserted on a stalk cell, terminal on the erect filaments, and lateral-basal to the multicellular (four to five celled) paraphyses (Fig. 4.21d). The unilocular structures were 6.3 – 11.0 µm in diameter and 16.3 – 28.3 µm in length while the paraphyses were 31.2 – 57.0 µm long. Cells of the paraphyses were 2.6 – 4.7 µm broad and 6.0 - 11.6 µm long. Hairs were not observed.

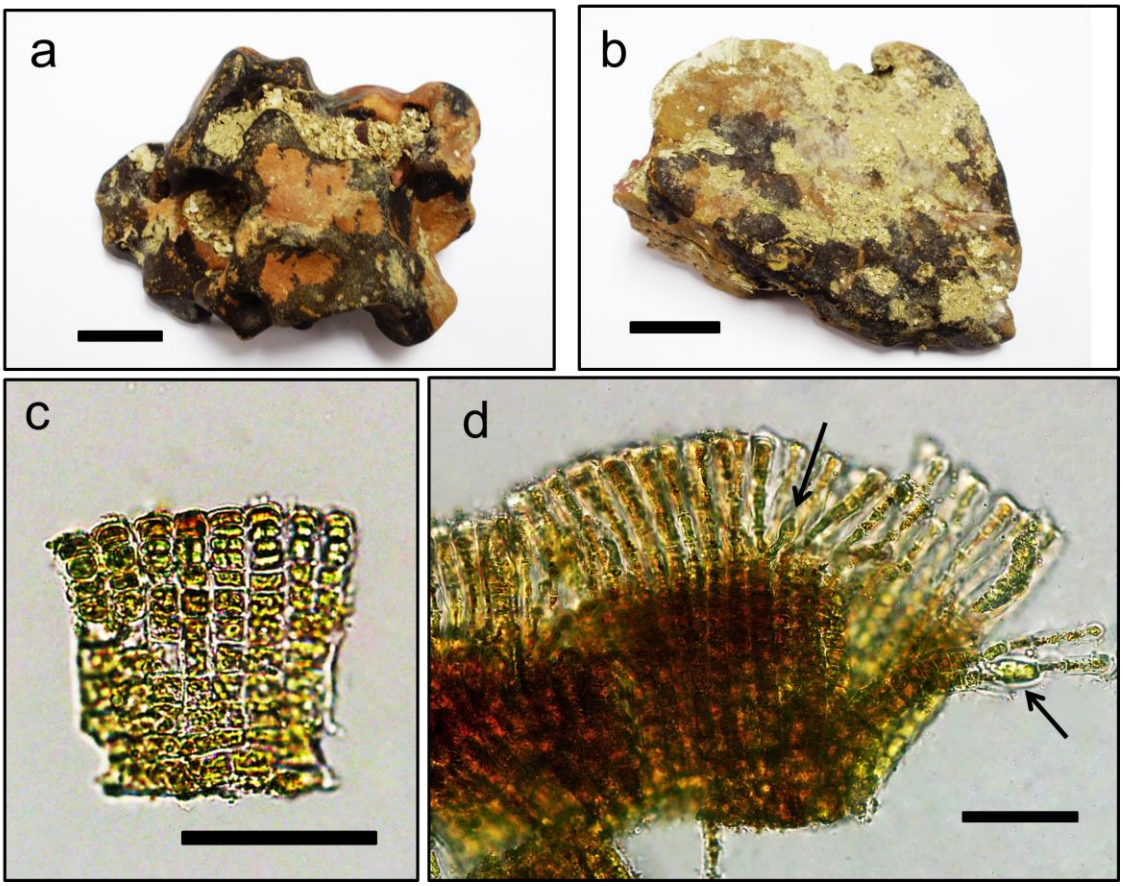

Figure 4.21 *Diplura* sp. 4 (a – b) Thalli on substratum [Voucher no. = PSM 12340 and PSM 12341, respectively] Scale bars = 2 cm. (c) Plurilocular bodies capped by a sterile terminal cell, borne at the apex of the erect filaments [Voucher no. = PSM 12347] (d) Unilocular reproductive structures (shown by arrows) borne lateral-basal to the paraphyses, terminal on the erect filaments. Scale bars = 50µm.

4.3.4 *Neoralfsia expansa* (J. Agardh) Lim & Kawai ex Cormaci & Furnari, Fig. 4.22

Thallus thick and crustose, growing on rocks or dead shells, colour greenish brown to black, circular in outline when young which become irregular when old, and occasionally confluent (Figs. 4.22a – d). Fertile crusts were gelatinous upon contact with water. Specimens from Japan tended to have an eroded centre, but this was not observed in specimens from Malaysia and Lombok Island. Thallus first attached firmly to the substratum by its entire under surface, without rhizoids, later tended to be free at the margins. The under surface of the thallus was rust-coloured. A distinguishing character between this taxon and other species of crustose brown algae in this study is the symmetrically bilateral organisation of the filaments i.e. a central layer giving rise to assurgent bi-directional filaments, and the distinct delineation of cortical and medullary layers (Fig. 4.22e). The medullary layer was 118.6 – 131.7 µm thick, cells of this layer were 8.5 – 15.4 µm broad; the cortical layer was 41.8 – 65.5 µm thick and the cells were sometimes branched, 3.0 – 11.0 µm wide and 0.4 to 2.1 times as long as wide. The thallus was made up of compact and laterally cohesive pseudoparenchymatous tissue throughout. Unilocular reproductive structures were long and ellipsoidal, clavate or obovate, measuring 15.4 – 71.2 µm long and 5.9 – 22.3 µm broad. They were terminally inserted on three to six stalk cells, terminal on the cortical cells, lateral to the surrounding laterally-free and multicellular paraphyses which were approximately 135 µm long (Fig. 4.22f). Plurilocular bodies were rare and were found in only one specimen whereas unilocular structures were more frequently encountered. Plurilocular bodies were similarly formed above the cortical layer and composed of biseriate locules capped by a sterile terminal cell (Fig. 4.22g) and measured 4.5 - 6.5 µm broad and 24.8 - 29.8 µm in length. Hairs were not observed.

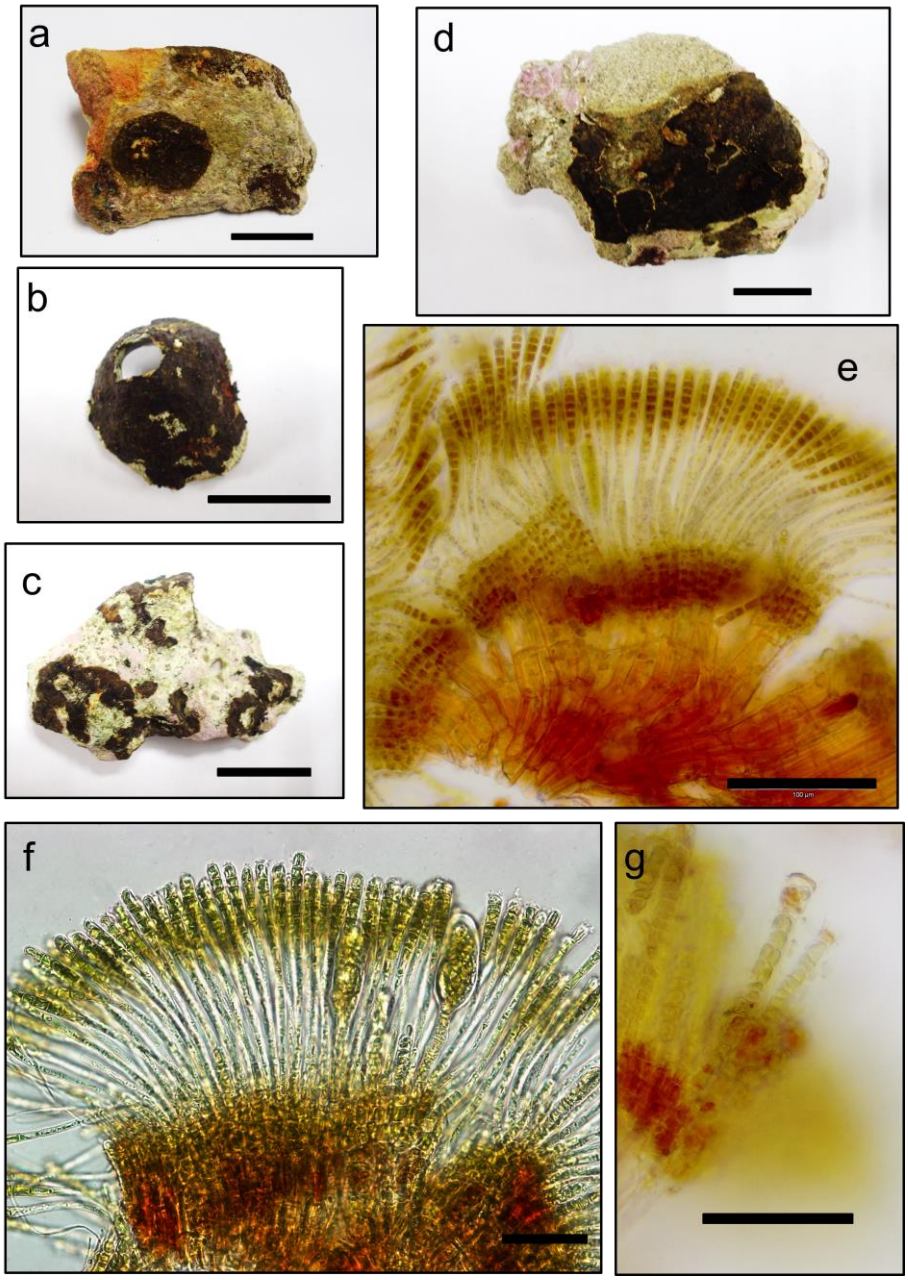

Figure 4.22 *Neoralfsia expansa* (a – d) Various morphologies of the crusts on substratum [Voucher no. = PSM 12342, PSM 12322, PSM 12249 and PSM 12247, respectively] Scale bars = 2 cm. (e) Distinct delineation of cortical and medullary layers, with unilocular structures accompanied by paraphyses [Voucher no. = PSM 12248] (f) Unilocular reproductive structures borne on stalk cells, lateral to the paraphyses and terminal on the erect filaments [Voucher no. = PSM 12223_72] (g) A biseriate plurilocular reproductive filament capped by a sterile terminal cell [Voucher no. = PSM 12247] Scale bars = 50 µm.

4.3.5 *Ralfsia* sp. 1, Fig. 4.23

Thallus dark brown to black, closely adherent to the substratum without rhizoids, almost circular to indefinite in outline and often coalescent with surrounding thalli (Fig. 4.23a). Mature thalli were crust-like and were easily removed from the substratum. Fertile portions of the thalli were especially mucilaginous. Thallus composed of prostrate filaments from which upwardly curved and strongly united assurgent filaments arise. The vegetative filaments consisted of approximately ten to 12 cells measuring 4.9 – 6.8 µm wide and 5.3 – 8.0 µm long (length to diameter ratio of 0.9:1 to 1.6:1). Unilocular reproductive structures were borne lateral to the paraphyses, either sessile or inserted on one stalk cell, and terminal to the erect filaments (Fig. 4.23b). The unilocular structures were globose to elliptical, measuring 7.0 – 21.4 µm broad and 13.5 – 62.9 µm long, whereas the clavate and multicellular paraphyses (consisting of five to nine cells) were 30.3 – 107.4 µm long and 2.3 – 9.1 µm broad. Plurilocular reproductive structures were not observed.

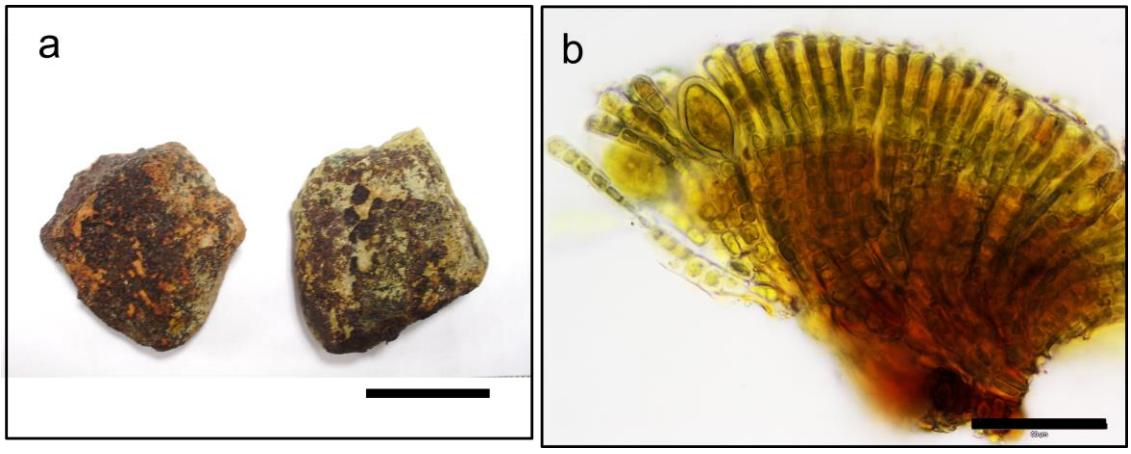

Figure 4.23 *Ralfsia* sp. 1 (a) Thalli on substratum [Voucher no. = PSM 12255 (left) and PSM 12256 (right)] Scale bar = 2 cm. (b) Unilocular reproductive structures borne on a stalk cell lateral to the paraphyses and terminal on the erect filaments [Voucher no. = PSM 12256] Scale bar = 50 µm.

4.3.6 Unidentified crustose brown alga species 1, Fig. 4.24

Thallus was thick, dark brown to black, initially circular in outline, later tended to be confluent with surrounding thalli and firmly attached to the substratum by whole under surface. Surface of the thallus ranged from velvety smooth to crusty with cracks all over the surface (Figs. 4.24a - d). Fertile portions of the thallus were gelatinous. Thallus comprised simple and unbranched erect filaments arising from a basal layer two to three celled thick. Erect filaments were rather tightly adjoined and consisted of ten to 20 cells, measuring 3.7 – 7.4 µm wide and 2.8 – 13.3 µm long, the length to diameter ratio was 0.7:1 to 3.2:1. Unilocular reproductive structures were terminally borne on the erect filaments, superficial to the surrounding filaments, unaccompanied by paraphyses, cylindrical and measured 4.3 – 5.9 µm wide and 20.5- 24.2 µm long (Figs. 4.24e - f). These structures were formed via differentiation of the terminal cells. Plurilocular structures were unknown. Hairs were not observed.

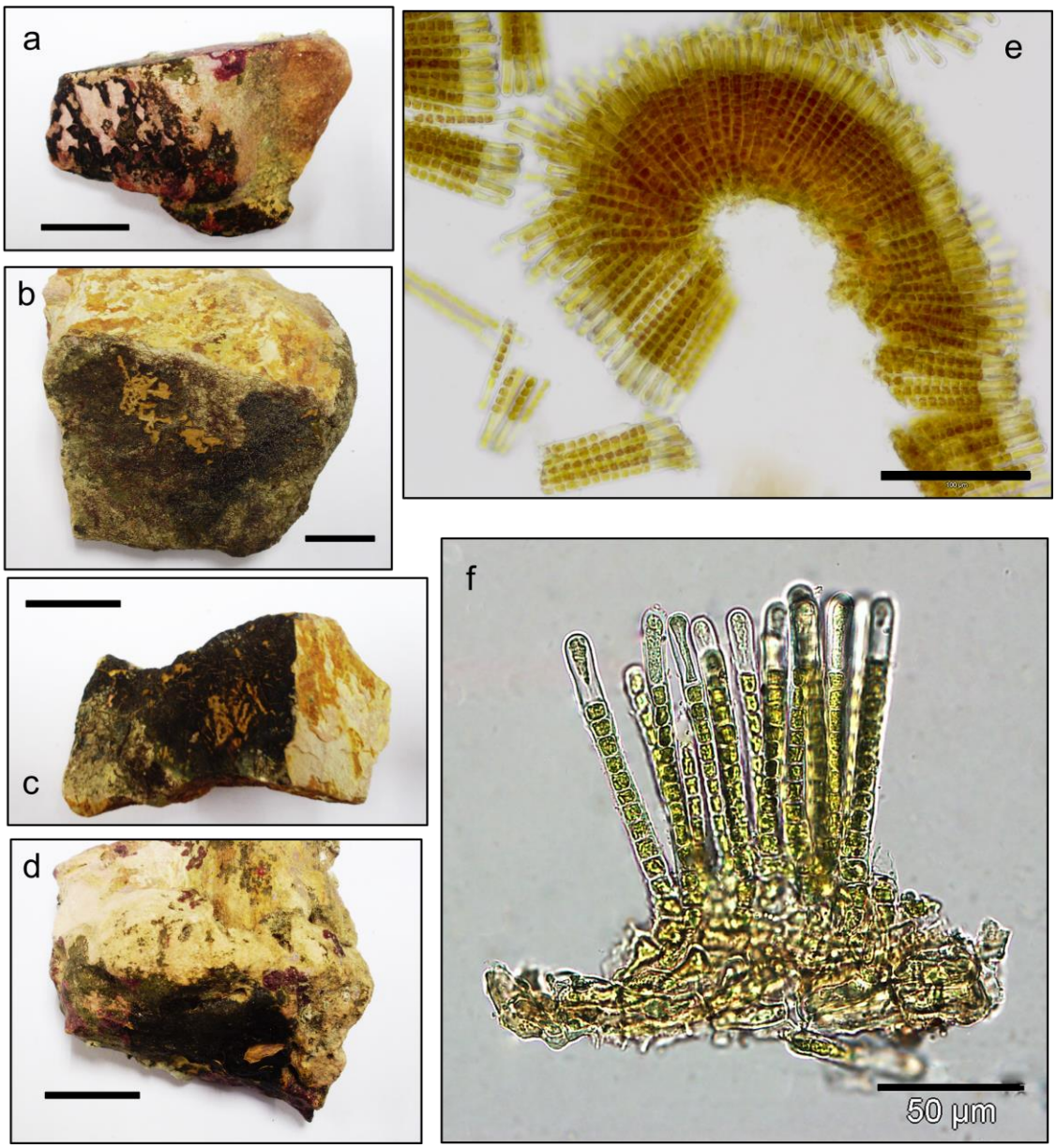

Figure 4.24 Unidentified crustose brown alga species 1 (a – d) Varioius morphologies of thalli on substratum. [Voucher no. = PSM 12190, PSM 12176, PSM 12174 and PSM 12195, respectively] Scale bars = 2 cm. (e) Laterally cohesive erect filaments with terminally inserted unilocular structures [Voucher no. = PSM 12190] Scale bar = 100 µm (f) Unilocular reproductive structures terminally borne on the erect filaments [Voucher no. = PSM 12190] Scale bar = 50 µm.

4.3.7 Unidentified crustose brown alga species 2, Fig. 4.25

Thallus was dark brown to black. Other morphological features of the thallus could not be determined as the sole specimen collected was of scrapings from the substratum at the site of collection. Thallus comprised laterally free erect filaments (cells near the basal layer were more closely adjoined to each other), approximately 275 µm long, arising from a distromatic basal layer (Fig. 4.25a). The erect filaments were relatively long, consisting of 30 to 40 cells per erect filament. Cells at the distal end measured 3.0 – 5.1 µm wide and 4.1 - 7.7 µm long (length to diameter ratio of 0.8:1 to 2.4:1) whereas cells near the basal end measured 4.5 – 6.9 µm wide and 5.3 – 7.7 µm long (length to diameter ratio of 0.9:1 to 1.6:1). Plurilocular reproductive structures appeared to be developing near the apex of the erect filaments in which some cells were seen dividing horizontally and some, vertically (Fig. 4.25b). Cells of the basal layer were 9.4 – 14.1 µm wide and 3.9 – 4.2 µm long (Fig. 4.25c). Unilocular reproductive structures were not observed. Hairs emerging from the basal layer were occasionally observed.

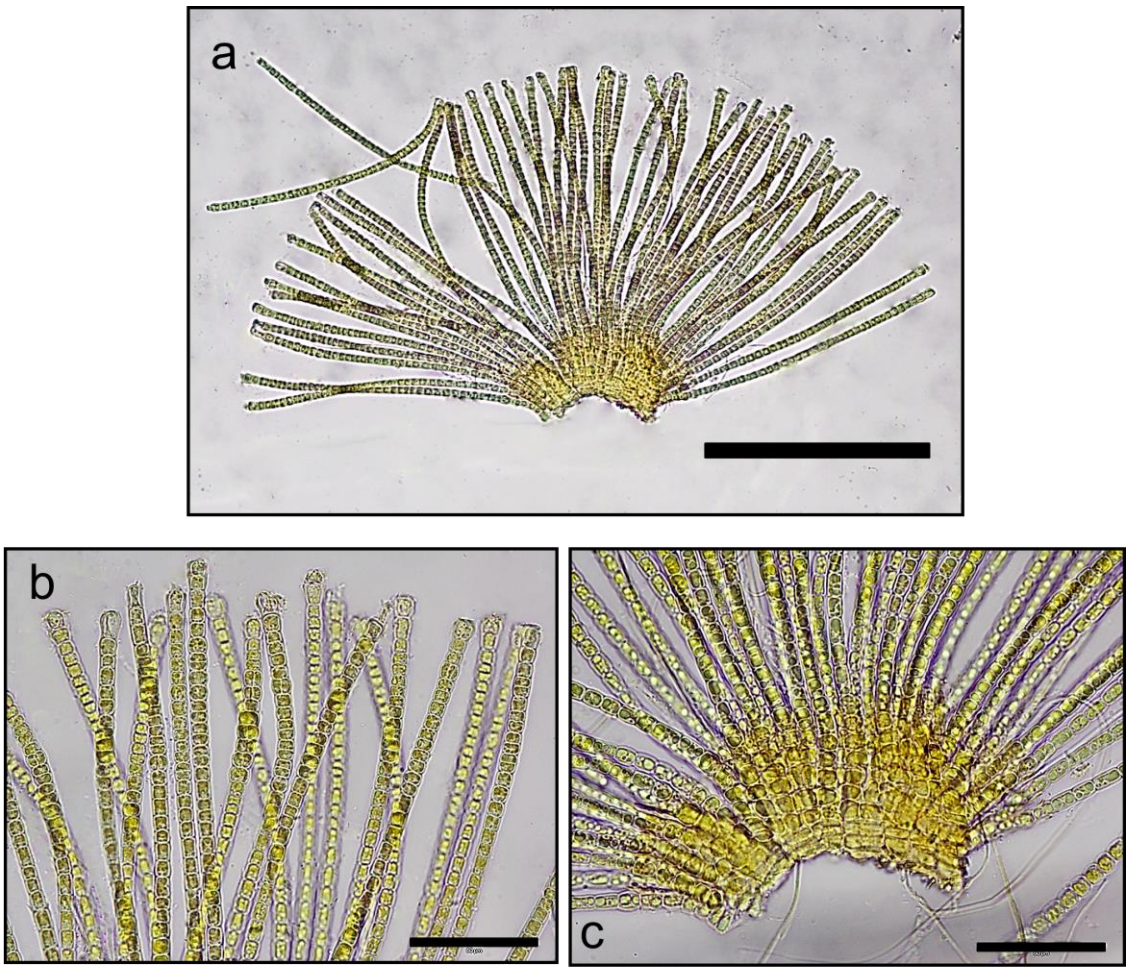

Figure 4.25 Unidentified crustose brown alga species 2 [Voucher no. = PSM 12217] (a) Laterally free erect filaments arising from a basal plate. Scale bar = 200 µm. (b) A zoomed-in view of cells at the distal end of the erect filaments (c) A zoomed-in view of cells at the basal end of the erect filaments. Scale bars = 50 µm.

4.3.8 *Hapalospongidion saxigenum* Lindauer, Fig. 4.26

Thallus smooth, olive-green in colour, gelatinous upon contact with water and firmly adherent to the substratum without rhizoids (Fig. 4.26a). Thallus consisted of long erect filaments (400 - 600 µm) arising from a basal plate several cells thick. The erect filaments were simple, narrowly clavate, sometimes branched near the basal layer, laterally free and held together by mucilaginous material, comprising approximately 40 - 60 cells (Figs. 4.26b - c). Cells of the erect filament were 3.7 - 8.1 µm wide and 6.3 - 12.9 µm long, with a length to diameter ratio of 0.9:1 to 3.2:1. Lower cells of the erect filaments were cylindrical while the upper cells were more rounded. Unilocular reproductive structures were terminally inserted on long stalks (approximately 20 to 35 cells) of which the cells were morphologically different (larger in dimension) from those of the erect filaments, (probably) arising directly from the basal plate, lateral and middle or sub-superficial to the surrounding filaments and unaccompanied by paraphyses (Fig. 4.26d). The unilocular structures were elliptical and relatively large, measuring 9.2 - 31.2 µm broad and 44.8 - 177.6 µm long. The stalk cells measured 6.8 - 9.4 µm in diameter and 5.4 - 17.2 µm long, with a length to diameter ratio of 0.7:1 to 2.5:1. Plurilocular reproductive structures were not observed. Hair tufts were occasionally present.

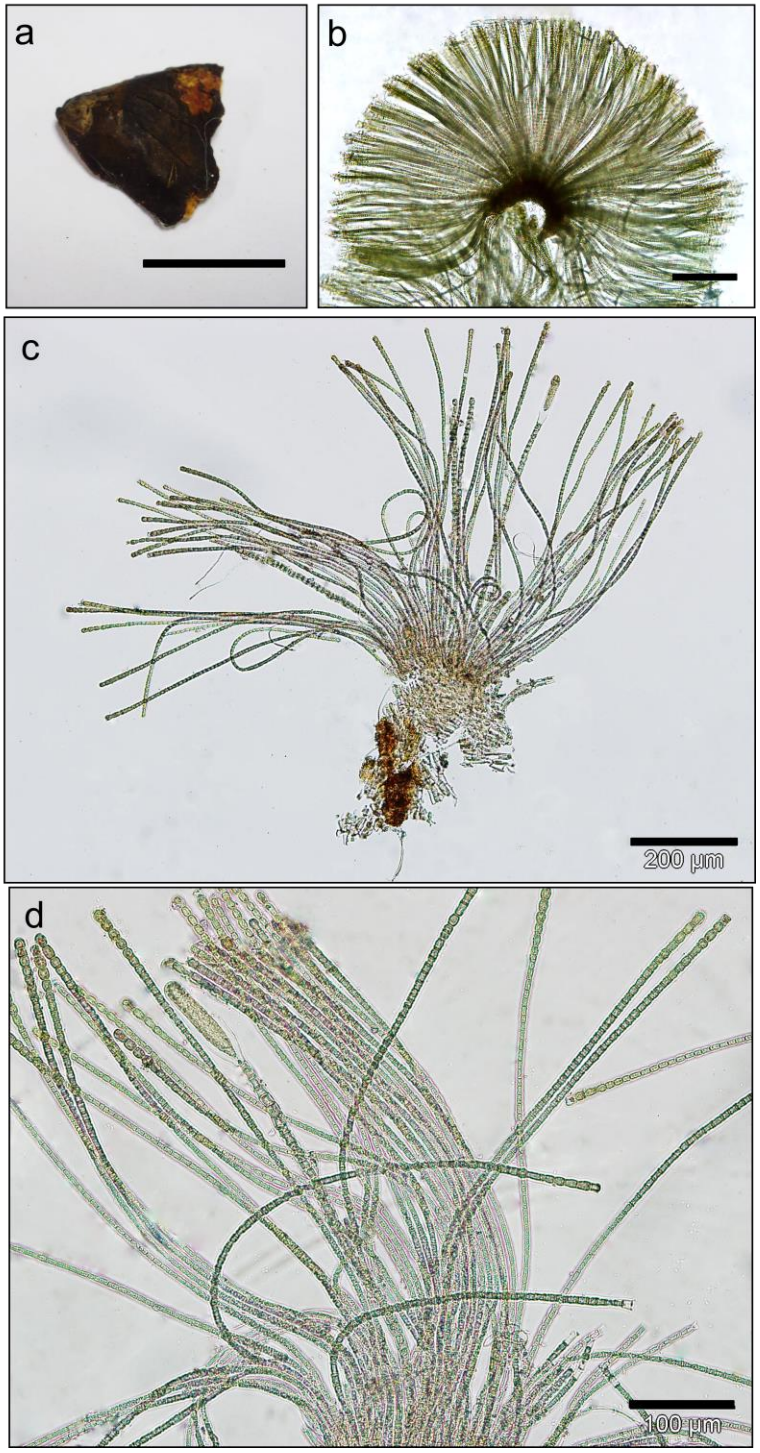

Figure 4.26 *Hapalospongidion saxigenum* [Voucher no. = ASL 418] (a) Morphology of the epilithic crust. Scale bar = 1 cm. (b) Laterally free erect filaments spread out in a fan shape. (c) Conspicuously long erect filaments with a large number of cells arising from a basal plate. Scale bars = 200 µm (d) A unilocular reproductive structure terminally inserted on a long stalk, lateral to the surrounding filaments. Scale bar = 100 µm.

4.4 Simplified floristic results of crustose brown algae from Malaysia and Lombok Island

As shown in Table 4.2, *Mesospora schmidtii* is the most common brown crusts found in Malaysia with 45 specimens, *M. negrosensis* (8), *M. elongata* (1), *Mesospora* sp. C (6), *Mesospora* sp. D (3), *Mesospora* sp. 1 (7), "*Mesospora*" sp. 3 (4), *N. expansa* (3), *Diplura* sp. 1 (14), *Diplura* sp. 3 (6), *Diplura* sp. 4 (6) and the unidentified crustose brown alga species 1 (8). *Mesospora* sp. 2, *Diplura* sp. 2 and the unidentified crustose brown alga species 2 were not found in Malaysia. Meanwhile, there were only two *M. schmidtii* among the specimens collected from Lombok Island. The breakdown of specimen number per species are as follows: *M. elongata* (4), *Mesospora* sp. D (1), *Mesospora* sp. 1 (3), *Mesospora* sp. 2 (6), "*Mesospora*" sp. 3 (5), *N. expansa* (5), *Diplura* sp. 1 (1), *Diplura* sp. 2 (7), *Diplura* sp. 3 (6) and the unidentified crustose brown alga species 2 (1). *Mesospora negrosensis*, *Mesospora* sp. C, *Diplura* sp. 4 and the unidentified crustose brown alga species 1 were not found on Lombok Island. The crustose brown algae were generally found at the mid- and upper intertidal levels, apart from *N. expansa* which were occasionally found at the lower intertidal level. As most of the samplings were conducted at the mid- and upper intertidal levels, this may have contributed to the low number of *N. expansa* samples collected.

A relatively higher diversity of crustose brown algae was recorded at the southwest and southeast coasts of Peninsular Malaysia, east coast of Sabah and west coast of Lombok Island. *Mesospora schmidtii* was present at the south west and east coasts (the term "coast" herein refers to both mainland coasts and offshore islands, if applicable) of Peninsular Malaysia, east coast of Sabah and was collected only at one site (Batukijok) on Lombok Island. *Mesospora elongata* was found only at the west coast of Lombok Island while one collection was made from the east coast of Sabah.

Mesospora negrosensis can be found at the south and east coasts of Peninsular Malaysia and east coast of Sabah. *Mesospora* sp. C was collected only from the southwest and southeast coasts of Peninsular Malaysia. *Mesospora* sp. D was present at the south coast of Peninsular Malaysia and one sample was obtained from Batu Layar, Lombok Island. *Mesospora* sp. 1 was found on the east coast of Peninsular Malaysia and west coast of Lombok Island. *Mesospora* sp. 2 was collected only from Lombok Island whereas "*Mesospora*" sp. 3 was distributed at the east coast of Sabah and the east and west coasts of Lombok Island. Specimens of *Neoralfsia expansa* were recorded at the southwest and east coasts of Peninsular Malaysia and west coast of Lombok Island. *Diplura* sp. 1 was present at the southwest and east coast of Peninsular Malaysia while the distribution of *Diplura* sp. 2 was limited to the east coast of Lombok Island. *Diplura* sp. 3 can be found at the southwest and east coasts of Peninsular Malaysia, east coast of Sabah and west coast of Lombok Island. *Diplura* sp. 4 was obtained from the northeast and south coasts of Peninsular Malaysia. The unidentified crustose brown alga species 1 was distributed at the south coast of Peninsular Malaysia. A single collection of the unidentified crustose brown alga species 2 was made from Batu Layar, Lombok Island.

Table 4.2 Floristic results of the 15 species identified in this study from Malaysia and Lombok Island with information on the range of distribution and number of sequences.

Species (number of specimens)	Range of collection (number of specimens per site)	Number of satisfactory sequence	
		*rbc*L	*cox*1-5'
M. schmidtii (n = 47)	MAL: CHE (5), MER (10), POR (5), PTL(1), DAN (2), TKA (2), TKS (4), TKR (1), SEM (15)	47	47
	LOM: BAT (2)		
M. elongata (n = 5)	MAL: SEM (1)	5	5
	LOM: NIP (1), GIL (2), BAT (1)		
M. negrosensis (n = 8)	MAL: CHE(1), MER (2), CHD (3), SEM (2)	8	8
Mesospora sp. C (n = 6)	MAL: POR (1), CHE (3), TKS (1), TKR (1)	6	6
Mesospora sp. D (n = 4)	MAL: CHE (2), MER (1)	4	4
	LOM: LAY (1)		
Mesospora sp. 1 (n = 10)	MAL: KEM (4), CHD (2), CHE (1)	10	10
	LOM: LEN (1), GIL (2)		
Mesospora sp. 2 (n = 6)	LOM: LAY (1), LAB (5)	6	6
"*Mesospora*" sp. 3 (n = 9)	MAL:MER (1), SEM (3)	9	9
	LOM:GIL (3), RAM (1), LAB (1)		
Neoralfsia expansa (n = 8)	MAL: BES(1), KEM(1), CHE (1)	8	7
	LOM: NIP (1), LAY(1), GIL(3)		
Diplura sp. 1(n = 15)	MAL: MER (1), POR (7), CHD (3), TKS (2), SEM (1)	14	11
	LOM: GIL (1)		
Diplura sp. 2 (n = 7)	LOM: LAB (5), RAM (2)	7	7
Diplura sp. 3 (n = 12)	MAL:CHE (1), MER (1), POR (3), SEM (1)	10	9
	LOM: LAY (2), GIL (4)		
Diplura sp. 4 (n = 6)	MAL: SAY (2), CHE (4)	6	6
Unidentified crustose brown alga species 1 (n = 8)	MAL: POR (2), CHE (3), MER (3)	8	7
Unidentified crustose brown alga species 2 (n = 1)	LOM: LAY (1)	1	1

Abbreviations for locations are: MAL, Malaysia; CHE, Pulau Che Kamat, Johor; MER, Pulau Merambong, Johor; POR, Port Dickson; PTL, Pelabuhan Tanjung Langsat, Johor; SEM, Semporna, Sabah; DAN, Kampong Dandulit, Sabah; BES, Pulau Besar, Melaka; CHD, Pantai Chendering, Terengganu; KEM, Pantai Kemasik, Terengganu; TKA, Telok Kalong, Terengganu; TKS, Teluk Sari, Johor; TKR, Teluk Ramunia, Johor; SAY, Pulau Sayak, Kedah; LOM, Lombok Island, Indonesia; NIP, Nipah; BAT, Batukijok; GIL, Gili Genting; LAY, Batu Layar; LAB, Labuhan Pandan; RAM, Rambang; LEN, Lendang Luar.

CHAPTER 5: DISCUSSION

5.1 Analysis of sequence data and molecular phylogenetics

All three methods of phylogenetic inference (ML, MP, BI) resulted in trees with near identical topology for all well-supported nodes for the three data sets (genes combined or separate). Nonetheless the concatenated data gave better resolution and clade support than each individual gene. The topologies obtained in the *rbc*L only and combined data analyses were reasonably congruent at the inter-ordinal level, although there was little resolution of relationships among the orders. Phylogenetic signal (at the species level) was virtually congruent between the more variable *cox*1-5' gene and the conserved *rbc*L gene and mainly carried by chloroplastic information. Missing *cox*1-5' sequences in the combined data set did not affect the overall phylogeny with variations only in the position of certain clades with low or no support.

5.1.1 Taxonomic position and relationships of crustose brown algae examined in this study

Results of this study corroborated with the findings by Lim et al. (2007) in which the crustose brown algae were shown to be polyphyletic, indicating that the crustose form has evolved several times among the brown algae and displayed convergence or homoplasy. Specimens examined in this study (Appendix A) were distributed in two to three clades and the Clade 1 elucidated in all the analyses constitutes a monophyletic group corresponding to the order Ralfsiales. The circumscription of Ralfsiales included the Ralfsiaceae, Neoralfsiaceae, Mesosporaceae and a possible new family. Meanwhile, the putative species of *Diplura* and the unidentified crustose brown alga species 1

(USP1) are shown to be early diverging lineages and along with several other crustose brown algae (*P. maculiforme*, *P. fluviatile*, *P. roscoffense* and *H. fluviatilis*) are phylogenetically distant from the Ralfsiales. Although the Ralfsiales was artificially resolved as the most derived lineage in the present analyses as a result of the taxon sampling, this order is always nested within the BACR and most of the time separated from the remaining crustose brown algae by the SSDO clade. Two anomalies were observed in the ML and BI analyses of the *cox*1-5' gene in which the "*Mesospora*" sp. 3 clade formed an unusually long branch and the grouping of *P. roscoffense* among the members of Clade 1 instead of its usual position in the lower half portion of the tree as seen in the *rbc*L and combined analyses. Reason for the long branching is not altogether clear although Draisma et al. (2001) stated that long terminal branches can be indicative of extinction (i.e. a surviving lineage that was once more speciose). Meanwhile, the USP1 which is always positioned in the basal part of the tree unusually formed a sister relationship with the Neoralfsiaceae in the MP analysis of the *cox*1-5' gene.

Seven species of *Mesospora* (*M. schmidtii*, *M. elongata*, *M. negrosensis*, *Mesospora* sp. C, *Mesospora* sp. D, *Mesospora* sp. 1, *Mesospora* sp. 2), four genetic species of *Diplura* (*Diplura* sp. 1, *Diplura* sp. 2, *Diplura* sp. 3 and *Diplura* sp. 4), a new genus tentatively designated "*Mesospora*" sp. 3, the monotypic genus *Neoralfsia expansa*, a *Ralfsia*-like species from North America and two undetermined crustose brown algae were identified from the molecular analyses of the specimens examined for this study. Provisional names i.e. *Mesospora* sp. 1, *Mesospora* sp. 2, "*Mesospora*" sp. 3, *Diplura* spp. 1 - 4, *Ralfsia* sp. 1, USP1 and the unidentified crustose brown alga species 2 (USP2) were assigned when specimens do not cluster with published sequences.

In the Mesosporaceae clade, seven genetic species each resolved with moderate to full support are recognised. Molecular analysis could only be carried out on three out of six currently recognised species of *Mesospora* i.e. *M. schmidtii*, *M. negrosensis* and

M. elongata, the latter being a recent addition to the genus. Molecular study was not accomplished for the rest of the members of *Mesospora* due to the difficulty in obtaining specimens from their type locality and information on the housing institutions of the types was lacking. Therefore, comparison was only made based on morpho-anatomical data from literature. The specimen GIL88, tentatively designated as *Mesospora* sp. 1 may be a separate species as shown by its somewhat distant relationship from other members of its subclade. Following Lim et al. (2007), a conclusion on the validity of Mesosporaceae is reserved pending the inclusion of the remaining genera of this family *viz*. *Basispora* and *Hapalospongidion*. However, the incorporation of a single *rbc*L sequence of *H. saxigenum* in the present study appears to support the validity of the family and the treatment of *Mesospora* and *Hapalospongidion* as distinct genera. The reduced bootstrap support obtained for the Mesosporaceae with the inclusion of *H. saxigenum* is believed to be a result of disproportionate taxon sampling. Incorporation of more sequences of *Hapalospongidion*, either of different species or from different geographic locations may alter the topology and/or nodal support of Mesosporaceae.

Each of the phylogenetic analyses demonstrated the separation of "*Mesospora*" sp. 3 from other species of *Mesospora* (except for *Mesospora* sp. G) and at times, its exclusion from the Mesosporaceae. The large genetic variation between "*Mesospora*" sp. 3 and other species of *Mesospora* (*rbc*L: 9.50 - 11.74%; *cox*1: 32.79 - 37.11%) seemed to warrant its recognition as an independent genus. This is comparable to the genetic divergence between other genus pairs, for example *R. fungiformis* and *N. expansa* which stands at 11.16 - 11.73% (*rbc*L) and 20.78 - 22.96% (*cox*1). The genetic divergences between "*Mesospora*" sp. 3 and its other sister taxa are as follows: *N. expansa*, 11.36 - 12.54% (*rbc*L) and 34.58 - 37.41% (*cox*1); *H. saxigenum*, 11.28 - 12.32% (*rbc*L); USP2, 12.48 - 13.09% (*rbc*L) and 35.62 - 36.81% (*cox*1); *A. japonicus*,

10.39 - 10.69% (*rbc*L) and 36.14 - 38.10% (*cox*1); *R. fungiformis*, 10.21 - 10.71% (*rbc*L) and 35.56 - 36.77% (*cox*1) ; *H. saxicola*, 11.06 - 11.51% (*rbc*L); *Ralfsia* sp., 11.73 - 13.38% (*rbc*L) and 34.87 - 36.66% (*cox*1). At this stage, the familial assignment of the supposed new genus (and the closely related *Mesospora* sp. G) is still uncertain although the possibility of establishing a new family should not be dismissed. This taxon consistently formed a close relationship with the Mesosporaceae and/or the Neoralfsiaceae and its inclusion in the Ralfsiales is without doubt.

Species of *Ralfsia* were polyphyletic. *Ralfsia* sp. 1 from North America did not group with members of the Ralfsiaceae which include both *R. fungiformis* and *R. verrucosa* (Lim et al., 2007) but it was instead nested in a separate clade consisting of two undescribed species of *Ralfsia* from New Zealand and Norway. Specific epithets were not applied to the *Ralfsia* species due to a lack of morpho-anatomical characters; sequencing of the many *Ralfsia* spp. reported from North America (see section 2.4.1.1) is likely to provide a positive identification of *Ralfsia* sp. 1. Incorporation of gene sequences from various other species of *Ralfsia* may also yield informative results on the relationship between the Ralfsiaceae and the *Ralfsia*-like species clade. As for the USP2, although it is consistently grouped within the Ralfsiales, it has no obvious affinities at the familial and generic levels.

Near the basal end of the phylogenetic trees, putative species of *Diplura* formed a close relationship with the order Ishigeales, similar to the results by Lim et al. (2007). Although the establishment of a new family as suggested by these authors is necessary to accommodate species of *Diplura*, it seemed premature to do so at this stage pending the publication of the gene sequences of the generitype, *D. simulans*. Further investigation of morpho-anatomical characters, life history and cytology will help in understanding the evolutionary history of this early diverging group. As of now, the placement of this genus among the early lineages of brown algae is supported by the

possession of several discoid chloroplasts per cell (Draisma, 2002) and a presumed isomorphic life history. Nevertheless, results of this study do not support the inclusion of *Diplura* in the Ishigeales as proposed by Lim et al. (2007). The Ishigeales is characterised by unilocular sporangia transformed from cortical cells and plurilocular sporangia lacking sterile terminal cells, features that are unrelated to *Diplura*. Investigation of *Diplura*'s plastid ultrastructure (probably devoid of pyrenoids like other basal phaeophycean lineages) may also yield informative results to help justify an order of its own. Putative *Diplura* spp. from Malaysia and Lombok Island displayed sister relationship with *D. simplex* and two undescribed *Diplura* spp. from Japan. The phylogenetic relationship inferred for species of *Diplura* examined in this study (except for *Diplura* sp. 3) mirrored the geographic location where these specimens originated, i.e. samples from Japan collectively formed a sister clade to Indo-Malaysian samples. *Diplura* sp. 3 which was resolved as a sister to other species of *Diplura* may represent a separate but closely related genus, nonetheless current morpho-anatomical data are insufficient to support this hypothesis. The possibility of *Diplura* spp. 1, 2 and 4 representing a single species should not be discounted at this stage. *Diplura* sp. 1 and *Diplura* sp. 3 were described as *Diplura* sp. F and *Diplura* sp. G, respectively in Poong et al. (2014).

The USP1 surprisingly formed a close relationship with *Porterinema fluviatile*, a predominantly endophytic or epiphytic crustose brown alga, despite its morpho-anatomical similarity to *Petroderma maculiforme*. Nonetheless, further information on this undescribed alga in particular its plurilocular reproductive structures (which may offer valuable insights on its identity) remain elusive. As such, no conclusion can be made on its taxonomic position at the generic and familial levels.

Effort to loan the types of *H. gelatinosum* and *D. simulans* from their housing institutions is still futile as the specimens were unsuccessfully located. This presented a

major setback to this study as it was far easier and cost effective to obtain herbarium specimens rather than obtaining fresh materials from diverse geographic localities, particularly when species are becoming extinct or increasingly rare in the wild (Särkinen et al., 2012). Additionally, the altered landscape of the type localities increases the difficulty of recollecting authentic specimens. It was unfortunate that an attempt by Dr John A. West (University of Melbourne) to collect the generitype of *Hapalospongidion* (*H. gelatinosum*) from its type locality i.e. Pacific Grove, California was not successful. Since genetic comparison between the type specimens and my own specimens could not materialise, the option available was to conduct detailed morphological comparisons using data from literatures. Nevertheless, the independence of *Mesospora* and *Hapalospongidion* appears valid as seen from the consistent separation of *H. saxigenum* from the *Mesospora* spp. in the phylogenetic results. Likewise, the establishment of a new family for members of *Diplura* is subjected to the availability of new authentic specimens of the type for molecular analysis.

In general, results of the molecular phylogenetic analyses from this study agree closely with the findings of several other published studies (McCauley and Wehr, 2007; Lim et al., 2007; Bittner et al., 2008; Phillips et al., 2008; Silberfeld et al., 2010) in which the crustose brown algae are distributed across different orders. Despite these studies having improved our understanding of the relationship between these simple brown algae, many of the crustose brown algal taxa discussed in Chapter 2 (*Lithoderma*, *Sorapion* and *Zeacarpa,* to name a few) are yet to be sequenced and these taxa are likely to be disassociated from the Ralfsiales when their DNA sequence data becomes available. The possibilities for a revised circumscription of the Ralfsiales should not be dismissed, as we anticipate the publication of sequence data of the many previously described taxa that was unsampled in the present study.

5.1.2 Comparison of performance between *rbc*L and *cox*1-5' markers

The choice of DNA marker for this study can be justified by the following reasons. The plastid-encoded *rbc*L gene has been one of the most widely used marker in the phylogenetic studies of brown algae noted for its higher resolving power (compared to rDNA), unambiguous alignment and relatively slow evolutionary rate (compared to mitochondrial-encoded markers) which makes it useful for ordinal and familial levels phylogenetic elucidation. The study by Lim et al. (2007) in particular provided a good data set of *rbc*L sequences of several crustose brown algal taxa. On the other hand, the *cox*1-5' marker was chosen for 1) its faster mutation rate to enable species level delimitation and 2) to include phylogenetic signals from both the plastid and mitochondrial genomes. This marker has also been tested for use as a barcode of red algae (Saunders, 2005; Robba et al., 2006), hence it was interesting to assess its applicability on the crustose brown algae. Several recent large scale phylogeny studies (e.g., Bittner et al., 2008; Silberfeld et al., 2010; Silberfeld et al., 2011) have included both *rbc*L and *cox*1-5' genes as part of a multi-locus approach to improve resolution.

The present molecular analyses involve a larger taxon sampling in which more brown algal orders were included compared to the study by Lim et al. (2007). Congruence tests were not conducted because some authors (e.g., Cho et al., 2004) have shown that statistically incongruent data can be combined for a better understanding of brown algal phylogeny, even if the genes employed possess different phylogenetic information. Although a number of taxa (e.g., *Mesospora* sp. D, *Ralfsia* sp. B) are missing in *cox*1 data, they were somehow included in the combined analyses since a study by Wiens (2009) demonstrated that the addition of missing taxa to a data set can be highly beneficial and improve phylogenetic accuracy and cases of decreased accuracy are limited. Phylogenetic analyses using *rbc*L data gave a satisfactory resolution at the ordinal and familial level whereas the use of *cox*1-5' data alone was

better suited for phylogeny elucidation at the species level. Intra-ordinal relationships were poorly resolved in the *cox*1 trees which were within expectation as the mitochondrial-encoded gene was known for its high evolutionary rate. This study also illustrated the feasibility of using *cox*1-5' as a barcode marker for species of crustose brown algae by means of displaying sufficient sequence diversity to enable discrimination of closely allied species, and yet there was no overlap between the intra- and interspecific divergences. Nonetheless, greater sampling effort needs to be undertaken to further assess the barcoding success of this marker. In the present study, the plastid marker used was also able to uncover cryptic species, as shown previously in the brown alga, *Scytosiphon lomentaria* (Cho et al., 2007). Nevertheless, the use of *cox*1 as a standard universal barcoding marker is advocated as this allows direct comparison of the material being studied across wide geographical boundaries (Robba et al., 2006). A combination of *rbc*L and *cox*1-5' data is suitable for use in species identification and phylogenetic reconstruction of the crustose brown algae.

Amplification and sequencing of *cox*1-5' gene sequence for *H. saxigenum* failed despite repeated attempts. The presence of large intron in the *cox*1-5' region which has been documented in other groups of algae (Watanabe et al., 1998; Ni-Ni Win et al., 2011; Kucera and Saunders, 2012) may in part explain the lack of success in amplification for *H. saxigenum* despite efforts in development of primers. This further strengthens the recognition of *Hapalospongidion* and *Mesospora* as distinct genera.

5.2 Morphological and anatomical studies

Species identification of crustose brown algae is challenging due to the paucity of morphological and anatomical characters. Presence of reproductive structures (ideally both unilocular and plurilocular ones) are extremely important in the identification of

these algae. However, collection of fertile specimens is often by chance, especially in the tropics where seasonality is not observed. Comparative studies of these algae are also confounded by variations in descriptive terminology of the position of reproductive structures (León-Alvarez and Norris, 2005; see section 2.5) and inadequate information of previously described species. Additionally, there is a risk of misidentification due to confluence of thalli from two or more different species. The homoplasy of the crustose form in brown algae as discussed above further increases the complexity of the taxonomy and phylogeny of this group of algae. Draisma et al. (2001) added that traditionally used taxonomic criteria such as modes of growth, type of life history and morphological grades of organisation (simplicity or complexity) are no longer useful in the phylogenetic sense as they have evolved and/or lost independently and repeatedly in the Phaeophyceae.

Colour of the crust is not a good criterion for identification of crustose brown algae, especially in the field. For the uninitiated, crustose red algae are easily mistaken for crustose brown algae in the field because the living thalli of the former appear brown in colour and only turn red upon drying. Meanwhile, crustose brown algae appear as dark brown to blackish crust in the field and become light to dark brown upon drying. However, patches of blue green algae on basalt rocks may also appear as blackish crust. Thus, a good practise to increase the probability of collecting genuine brown crusts is to scrape off some crust from the substratum using a blade and rub it in between our fingers to determine its actual colour (green, dark red-brown or dark brown-black). Taxa such as *Mesospora* and *Diplura* are easily recognised as their thalli (especially fertile ones) become gelatinous upon contact with water. Species of *Diplura* in this study are commonly found alongside the red crustose algae.

A number of morpho-anatomical features have been used as taxonomic characters at the family, genus or species levels by various authors and these included

the colour, diameter and thickness of crust; organisation of vegetative filaments (bilateral vs. unilateral symmetry, degree of lateral cohesion between filaments, demarcation of cortical and medullary layers); presence or absence of hair and/or rhizoids; number and morphology of plastids; absence or presence of pyrenoids; size and position of reproductive structures; presence or absence of paraphyses; structure of reproductive sorus (diffused vs. combined); number of sterile terminal cells (of plurilocular bodies) and number of stalk cells (of unilocular reproductive structures). However, not all of them are reliable for use as diagnostic features. I agree with Wilce et al. (1970) who stated that the use of crust colour, lateral dimensions or thickness as systematic criteria is not justified. The morphological variations of *N. expansa* crusts as seen in this study resulted from environmental conditions such as the depth and type of substratum. Morphology of the crust may be more useful in identifying species from the cold water region, for example *R. fungiformis* can be distinguished from *R. verrucosa* by the former's imbricated lobes while the latter is known for its verrucose thallus. This criterion is not as useful for tropical brown crusts which are mostly thin and dark-coloured.

Wilce et al. (1970) emphasised plastid morphology and number (which they consider to be a constant feature, at least in the uppermost two to three cells of each filament, except through aging) as critical taxonomic criteria for generic placement. The use of plastid number and shape for familial or generic delimitation is controversial due to inconsistencies observed by different authors for the same species. While most authors make use of this character (e.g., John and Lawson (1974) and Rull Lluch (2002) to distinguish *Basispora* from *Mesospora* and *Hapalospongidion*), others for instance Womersley (1987) consider plastid number to vary with the size and age of cells. Although I did not carry out detailed investigation of plastid number in my specimens, I am of the opinion that number and morphology of plastids and the presence or absence

of pyrenoids may serve as reliable taxonomic characters but careful attention has to be given to avoid mistaking physodes as plastids or better still to utilise TEM (Sanders et al., 2005) to accurately determine the number of plastids and presence or absence of pyrenoids.

Size of the unilocular sporangia which are frequently used as a diagnostic criterion for species delineation in *Ralfsia* may vary depending on the maturity of the structures and also on the ecological conditions (Tanaka and Chihara, 1980a). Variations in the size of the unilocular sporangia were also observed in *M. macrocarpa* by Feldmann (1937). Plurilocular reproductive structures are common in species of *Mesospora* and *Diplura*, but the opposite was observed for *N. expansa*. The lack of plurilocular reproductive structures can be interpreted as either failure of meiosis to occur in the unilocular reproductive structures or that the thallus in question represents the sporophytic stage of a heteromorphic brown alga (Hollenberg, 1968).

5.2.1 Intergeneric morpho-anatomical distinction

The three genera commonly found in this study i.e. *Mesospora*, *Diplura* and the monotypic *Neoralfsia expansa* can be distinguished based on a combination of characters which include relative thickness of thallus/crust, organisation of vegetative filaments, reproductive structures and number of chloroplasts (Table 5.1). Species of *Mesospora* are generally characterised by their gelatinous thallus, loose organisation of vegetative filaments (either partially or along the entire length of the filaments), single chloroplast in each cell, unilocular reproductive structures unaccompanied by paraphyses and plurilocular reproductive structures terminated by more than one sterile cells. The crusts of *Neoralfsia expansa* are characteristically thicker than *Mesospora* and *Diplura* and a distinct delineation of the cortical and medullary layers are observed.

Table 5.1 Intergeneric comparison between three crustose brown algal genera commonly found in Malaysia and Lombok Island

Genus	Relative comparison of thalli thickness	Organisation of vegetative filaments	Distinct delineation of cortical and medullary layer	Plurilocular reproductive structures	Unilocular reproductive structures	Number of chloroplasts per cell
Mesospora	Intermediate	Loosely adherent (partially or along entire length), with exception in certain species. Cell numbers of the vegetative filaments can be used for species level identification	No	Uni- or biseriate and terminated by one to four sterile cells. Generally more common than unilocular reproductive structures	Position of the structure on the erect filament (middle or basal) and number of stalk cells (usually one to two, can go up to 10 in *M. elongata*) is useful for species level identification. Lack paraphyses.	Single
Neoralfsia expansa	Thick	Filaments are tightly adherent with bilateral symmetry	Yes	Biseriate, terminated by a sterile cell. Less common than unilocular rep. structures	Terminally inserted on 3-6 stalk cells at the terminal end of erect filaments,basal-lateral to the paraphyses	Single
Diplura	Thin	Filaments are somewhat tightly adherent	No	Initially uniseriate later biseriate, and both rows of loculi shared a sterile terminal cell	Not observed in *Diplura* sp. 1. Generally inserted terminally on one to two stalk cells, basal-lateral to the paraphyses	Multiple

Species of *Diplura* are recognised by their relatively thin thallus, multiple chloroplasts per cell and plurilocular structures terminated by a single sterile cell.

Despite their current status as synonyms, the genera *Hapalospongidion* and *Mesospora* can be distinguished not only from a molecular basis, but also on morphological grounds. The former is easily differentiated from the latter by its substantially higher number of cells in the erect filament and a longer stalk supporting the unilocular reproductive structures (see section 5.2.1.1 for more details).

"*Mesospora*" sp. 3 is provisionally associated with *Mesospora* because of its thin thallus and the lack of true *Ralfsia* species from the Indo-Malay region. However, its anatomy which includes somewhat laterally cohesive erect filaments; unilocular reproductive structures inserted terminally on stalk cells, lateral at the base of paraphyses and intercalary plurilocular reproductive structures are similar to *Ralfsia*. Cell ultrastructure and life history patterns have not been elucidated for the putative new genus although both unilocular and plurilocular reproductive structures were observed. The morpho-anatomical features of this taxon do not fit with any known crustose brown algal genus except for *Ralfsia* (see Table 5.2). Among species of *Ralfsia*, "*Mesospora*" sp. 3 may be associated with *R. confusa* which has strictly erect filaments, unilocular reproductive structures sessile or on 1 - 3 (-6) celled-stalks and mostly uniseriate plurilocular reproductive structures. Most of the currently recognised species of *Ralfsia* possess slightly assurgent filaments; species with strictly erect (and sometimes loosely associated) filaments such as *R. clavata* (with multiseriate plurilocular bodies not terminated by sterile cells) and *R. californica* (unilocular sporangia sessile and plurilocular bodies unknown) have been shown to be crustose stages of the Scytosiphonaceae. Molecular phylogeny of this study has clearly indicated that "*Mesospora*" sp. 3 belongs to the Ralfsiales. Thus, this taxon may be recognised at the genus level via a combination of characters which include strictly erect vegetative

filaments comprising somewhat isodiametric cells; unilocular reproductive structures inserted on one to two-celled stalks, lateral at the base of paraphyses and mostly uniseriate plurilocular reproductive structures terminated by a sterile cell.

Table 5.2 Morpho-anatomical comparison of the various crustose brown algal genera

Genera	Unilocular rep. structures	Plurilocular rep. structures	No. of chloroplast and/or shape. Presence/absence of pyrenoids	Distinctive generic character(s) or additional information
Acrospongium	Terminally inserted on short filaments, sometimes intercalary,lack paraphyses	Unknown	Single plate-like plastid without obvious pyrenoid	Laterally free erect filaments
Analipus	Arise from the basal parts of paraphyses	Uni- or more frequently biseriate, with one to three sterile terminal cells.	Single parietal cup-shaped chloroplast without pyrenoid	Both the gametophyte and sporophyte stages are in erect form
Basispora	Terminal on long stalks from near the base of the vegetative filaments, without paraphyses	Unknown	Several discoid chloroplasts per cell, pyrenoid not mentioned	Simple, often assurgent laterally free filaments
Diplura	Unknown, first reported in this study	Uni- or biseriate, terminated by a sterile cell	Several per cell, pyrenoid not mentioned	Erect filaments simple and sparsely branched
Endoplura	Unknown	Initially uniseriate but later biseriate to quadriseriate , with 2 - 3 - (5) terminal sterile cells	Several per cell, pyrenoid not mentioned	Erect filaments firmly adjoined and infrequently branched
Hapalospongidion	Terminal on a long stalk or vegetative filament, not associated with paraphyses	Borne near the apex of erect filaments and usually multiseriate	Inconsistent reports by various authors, pyrenoid not mentioned	Erect filaments usually gelatinous and laterally free, with a higher number of cells in vegetative filaments than *Mesospora* and *Basispora*
Heribaudiella	Terminally inserted	Terminally inserted	Several, disc-shaped (reported to be single in the holotype)	Freshwater in habit, monostromatic basal layer from which dichotomously branched and laterally free erect filaments arise

Table 5.2 (continued)

Genera	Unilocular rep. structures	Plurilocular rep. structures	No. of chloroplast and/or shape. Presence/absence of pyrenoids	Distinctive generic character(s) or additional information
Heteroralfsia	Sessile on the basal cells of paraphyses which form from the cortical cells of the erect portions	Intercalary, terminated by a sterile cell	Single sinuate cup-shaped or several fragmented chloroplast without pyrenoid per cell	Erect sporophyte and crustose gametophyte
Jonssonia	Terminally inserted, sub-cylindrical or slightly clavate	Terminally inserted, articulated, uniseriate	-	Epiphytic. Firm, pulvinate, paren-chymatous thallus
Lithoderma	Yet to be determined	Laterally borne on filaments arising from the apical cell	Several per cell, pyrenoid not mentioned	Simple, closely packed (but laterally free according to Waern, 1949) and strictly erect filaments
Mesospora	Inserted on several celled stalk, lateral to the erect filaments, unaccompanied by paraphyses	terminated by one or several sterile cells	Single, pyrenoid not mentioned	Erect filaments usually gelatinous and laterally free
Nemoderma	Intercalary	Laterally inserted	Numerous discoid chloroplasts without pyrenoids	Isomorphic life history with marked anisogamy
Neoralfsia	Terminally inserted on a 3-6 celled stalk, lateral to the paraphyses	Biseriate and terminated by a sterile cell, near the apex of erect filaments	Single, lacking pyrenoids	Thallus with bilateral symmetry and distinct demarcation of cortical and medullary layers
Petroderma	Terminal on erect filament, without paraphyses	Terminal on erect filament, variable, uni- or multiseriate	Single plate-like plastid with a pyrenoid (can only be seen via TEM)	Soft crust, erect filaments gelatinous and loosely adjoined arising from a monolayer plate, euryhaline
Porterinema	Terminal on short erect filaments, without paraphyses	Intercalary, sessile or pedicellate (sometimes shaped like a crown)	One to several	Euryhaline in habit, peculiar plurilocular bodies, endophytic/ epiphytic/epilithic
Pseudolithoderma	Terminally borne on the erect filaments, unaccompanied by paraphyses	Terminally borne on the erect filaments, uni- or multiseriate	Several per cell, without pyrenoid	Simple, closely packed (may be laterally cohesive or free) and strictly erect filaments

Table 5.2 (continued)

Genera	Unilocular rep. structures	Plurilocular rep. structures	No. of chloroplast and/or shape. Presence/absence of pyrenoids	Distinctive generic character(s) or additional information
Ralfsia	Generally sessile or terminally inserted on a several celled-stalk (usually) lateral-basal to the paraphyses	Uni- or multiseriate, (usually) terminated by one or few sterile cells, borne near the apex of erect filaments	Usually single and plate-shaped, without pyrenoid	Closely packed erect or assurgent filaments not readily separating under pressure and form a pseudo-parenchymatous crust
Sorapion	Terminal, pear-shaped ,without paraphyses	Unknown	Single, plate-shaped (pyrenoid reported in *S. simulans*)	Erect filaments tightly adjoined
Symphyocarpus	Unknown	Terminal and multiseriate	Single, disc-shaped with a large pyrenoid	Generally epiphytic, erect filaments short and loosely united
Zeacarpa	Laterally inserted on uniseriate, free and fertile soral filaments	Unknown	Cells each with several chloroplasts without pyrenoids	Structure and disposition of unilocular sporangia distinctive
"Mesospora" sp. 3	Terminally inserted on a one celled-stalk, lateral-basal to the paraphyses	Mostly uni-seriate and terminated by a sterile cell?	Undetermined, appears to be single	Laterally cohesive and strictly erect filaments

Despite their sister relationship, "*Mesospora*" sp. 3 and *Mesospora* sp. G were anatomically different in terms of the latter's absence of paraphyses, plurilocular bodies terminated by three sterile cells and unilocular reproductive structures inserted on six-celled stalks. A detailed examination on *Mesospora* sp. G was not possible as the original specimen was unavailable for the present study.

Establishment of a new family in the Ralfsiales to accommodate the putative new genus appears justified from the elucidated phylogenies. This family, if valid, is distinguished from other families of the order on the basis of its structure of thalli and number of stalk cells of the unilocular reproductive structures (separating it from the Neoralfsiaceae) and unilocular reproductive structures accompanied by paraphyses (separating it from the Mesosporaceae). The clade "*Mesospora*" sp. 3 is persistently

resolved as a sister of the Neoralfsiaceae and Mesosporaceae. Despite its distant genetic relationship with the Ralfsiaceae, "*Mesospora*" sp. 3 is morpho-anatomically similar to *Ralfsia* and is only distinguished from the Ralfsiaceae by the thinner thallus and strictly erect filaments of "*Mesospora*" sp. 3. This taxon together with the *Ralfsia*-like species clade may be inferred as polyphyly of the genus *Ralfsia*.

Ralfsia sp. 1 collected from the Atlantic coast of North America, with its somewhat assurgent and tightly adherent filaments, and unilocular sporangia sessile or inserted on a single stalk cell is comparable to *R. hesperia*, *R. integra*, *R. endopluroides*, *R. occidentalis*, *R. pacifica* and *R. verrucosa* (the last three may be conspecific). Molecular data indicated the distant relationship between this species and *R. verrucosa*; it was instead closely related to two undescribed species (*Ralfsia* spp. B and C) which are unfortunately sterile but shared features such as tightly adherent filaments upwardly curved toward the surface. Like *Ralfsia* sp. 1, plurilocular reproductive structures are unknown for *R. hesperia* and *R. integra* whereas those of *R. occidentalis*, *R. pacifica* and *R. verrucosa* are reported to be terminated by a sterile cell; in *R. endopluroides*, the plurilocular bodies are biseriate and terminated by three sterile cells.

The terminal unilocular reproductive structures of USP1 suggest the affiliation of this taxon with members of the Lithodermataceae with the possible candidates being *Heribaudiella*, *Jonssonia*, *Petroderma*, *Porterinema*, *Pseudolithoderma* and *Sorapion*. In spite of its close relationship to *P. fluviatile* as elucidated from the molecular phylogeny; it differs from *P. fluviatile* in thalli construction and habit. The thallus of *Porterinema* is composed of loosely anastomosing and highly branched prostrate filaments, while the erect filaments are only a few cells in length. This is not true for USP1, in addition the characteristic plurilocular reproductive structures of *Porterinema* are not seen in USP1. *Porterinema* is reported to be largely endophytic or epiphytic and sometimes epilithic while specimens of USP1 are always epilithic. The unilocular

reproductive structures of USP1 are similar to those reported for *Petroderma* but the former is distinguished from the latter by its tightly adherent filaments and (seemingly) possession of several plastids per cell. Although USP1 is somewhat similar to *Sorapion* on the basis of its tightly adherent filaments and absence of plurilocular reproductive structures, it is distinguished from the latter which has a single plastid per cell and pear-shaped unilocular sporangia. USP1 is unlikely a species of *Heribaudiella* which are freshwater in habit. Among species of *Pseudolithoderma*, USP1 is anatomically similar to *P. subextensum* which was reported with laterally cohesive filaments not easily separated by pressure and terminal unilocular sporangia, elongated cylindrical often with waist. Plurilocular reproductive structures of USP1 which are yet to be observed may allow a better identification of this species. In terms of distribution, species of *Petroderma*, *Porterinema*, and *Sorapion* have only been reported in the cold waters of the Atlantic whereas some species of *Heribaudiella* and *Pseudolithoderma* were reported from the Indo-Pacific.

Identification of the single and sterile specimen of USP2 is difficult. Its laterally free erect filaments indicate its possible inclusion in *Acrospongium*, *Basispora*, *Mesospora* or *Hapalospongidion*. This taxon lacks unilocular reproductive structures and appears to be in the process of developing intercalary plurilocular bodies which are not reported in *Acrospongium* and *Basispora*. It is also shown to be molecularly distant from species of *Mesospora* and *Hapalospongidion*, the latter having a considerably larger number of cells per erect filament. USP2 was frequently resolved as a sister of the Neoralfsiaceae or *Ralfsia* sp. 1; nevertheless its anatomy is largely different from *N. expansa* and the putative species of *Ralfsia*. Further taxonomic conclusions on this taxon cannot be deduced at this stage; collection of fertile materials with both types of reproductive structures is necessary for positive identification.

5.2.1.1 Disagreement on the synonymisation of *Hapalospongidion*, *Mesospora* and *Basispora*

A review of literatures on the three genera indicated that they (at least *Mesospora* and *Hapalospongidion*) should be regarded as independent but closely related genera. Saunders (1899) described *Hapalospongidion* as having gelatinous thallus comprising erect, unbranched filaments arising from a distromatic basal plate. There are two types of vegetative filaments in which the intercalary plurilocular sporangia are formed on the longer vegetative filaments whereas unilocular sporangia are terminally inserted on the shorter filaments. According to Hollenberg (1942), plurilocular sporangia of *H. gelatinosum* occur on plants distinct from those bearing unilocular sporangia, but then again both types of plants are often indistinguishable due to thallus confluence. Saunders (1899) however illustrated both types of reproductive structures on the same thallus. The number of chloroplasts was not given particular mention in the original description although in absence of contrary statement, appeared to imply more than one chloroplast in each cell. The illustration by Saunders (1899: Pl. I, Fig. 2b and 2b') showed two peculiar structures on the shorter filaments: one was a series of enlarged cells which the author described as rare and treated as immature or abortive unilocular sporangia while the other was a few lateral outgrowths at the apex of the filament. However, Hollenberg (1942) commented that his specimens, collected from the type locality did not show any seriate intercalary unilocular sporangia as reported by Saunders (1899) and dismissed them as abortive plurilocular sporangia. Furthermore, he interpreted the few lateral outgrowths that were observed in his specimens as abortive accessory unilocular sporangia or the true tip of the fruiting filament. It should be noted that these structures have neither been reported in *Mesospora* nor in *Basispora*.

Morphology-wise, the soft and cushion-like thallus of *Hapalospongidion* is different from that of *Mesospora* which is hardly cushion-like. *Hapalospongidion* and *Mesospora* can be easily distinguished based on their number of cells of the erect filaments. Erect filaments of *Mesospora* are considerably shorter (less than 35 cells) as acknowledged by Womersley (1987) compared to *Hapalospongidion* whose erect filaments comprised 40 - 60 (-80) cells. Another distinction between *Mesospora* and *Hapalospongidion* is in the stalks where the unilocular reproductive structures are borne. In *Hapalospongidion*, unilocular structures are terminally inserted on long stalks (described by Saunders as shorter erect filaments) arising from near the basal layer. Saunders (1899) did not mention the number of cells of the stalks for *H. gelatinosum* while Lindauer (1949) mentioned that the stalks for *H. saxigenum* were of 12 cells or more. In contrast, unilocular reproductive structures are terminally inserted on considerably shorter stalks of less than 12 cells, issuing laterally (and not necessarily basal in position) from erect filaments in *Mesospora.* In this study, the stalks of *H. saxigenum* consisted of 20 to 35 cells. Like *Mesospora*, *Basispora* is distinguishable from *Hapalospongidion* on the basis of their number of cells of the erect filament and the number of stalk cells. The type species of *Basispora* is reported to have vegetative filaments of up to 40 cells and stalks consisting of (4-) 6 - 10 (-15) cells.

Although John and Lawson (1974) treated the three genera as distinct entities, their transfer of *H. saxigenum* to *Basispora* was unwarranted. The authors mentioned that they examined the type specimen of *H. gelatinosum* and *H. saxigenum* but not the other New Zealand species which was presumably *H. durvilleae*, but the figures that these authors cited from Lindauer (1949) do not seem to correlate with their statement. The justification for the transfer was multiple chloroplasts rather than a single one in each cell and that the unilocular sporangia were borne on distinct stalks arising from near the base of the erect filaments in *H. saxigenum*. Buchanan (2005) considers the

multiple plastids observed by Lindauer (1949) to be physodes misidentified as plastids as the New Zealand specimens he examined possess only a single plastid in each cell. Rull Lluch (2002) shares a similar opinion for the case of *Basispora*. The number of chloroplasts is an example of the inconsistency in morphological observations by different authors for a particular species. I disagree with John and Lawson (1974) and Tanaka and Chihara (1982) on their interpretation of stalks of the unilocular sporangia in *Hapalospongidion* as indistinguishable from ordinary vegetative erect ones. A careful review of Saunders's (1899) description of the longer and shorter erect filaments would highlight the difference of cell dimensions between the two types of filaments. Furthermore, my observation of *H. saxigenum* showed stalks morphologically differentiated from the vegetative erect filaments. However, it is unclear whether these stalks originate laterally from the base of erect filaments or were inserted directly on the basal plate.

Rull Lluch (2002) disagreed with the merger proposal by Womersley (1987) and commented that *Basispora* should be kept separate until the number and morphology of chloroplasts are elucidated although the author considered *Mesospora* to be synonymous with *Hapalospongidion*. According to Rull Lluch (2002), *Hapalospongidion* is characterised by presence of one to several blade-like chloroplasts rather than several discoid chloroplasts as originally ascribed to *Basispora* by John and Lawson (1974). Based on our literature review, *Basispora* bears a close resemblance to *Mesospora* in terms of their structure of thallus and reproductive structures, with the number of chloroplasts as the discerning factor. Species of *Mesospora* are generally reported with a single chloroplast whereas *Basispora* was reported to have several discoid chloroplasts in each cell. It is possible that physodes are mistaken as the multiple chloroplasts observed by John and Lawson (1974) in *Basispora*. Buchanan

(2005) went further by suggesting the merger of another genus, *Acrospongium* with *Hapalospongidion*.

On the basis of the above considerations, the merger of *Mesospora* and *Basispora* with *Hapalospongidion* as proposed by Womersley (1987), León-Alvarez and González-González (1993) and Parente et al. (2006) should be withhold until proven otherwise through molecular studies. Results from molecular analyses of this study which included *H. saxigenum* clearly indicate that *Mesospora* and *Hapolospongidion* should be treated as separate genera. According to Buchanan (2005), *H. saxigenum* is identical to *H. gelatinosum* as no differences were observed between the New Zealand material and the description of the type specimen of *H. gelatinosum*. I agree with Buchanan (2005) that the characters to distinguish the two species namely absence of plurilocular structures and poorly developed basal layer in *H. saxigenum* were insufficient, and the two are potentially conspecific. If this is the case, the molecular analysis incorporating *H. saxigenum* clearly showed that *Mesospora* and *Hapalospongidion* are distinct genera and thus the proposed synonymisation by Womersley (1987) is invalid. On morphological grounds, the two genera can be differentiated by the significantly larger number of cells (consequently, longer erect filaments) in *Hapalospongidion* (up to 60 cells) as opposed to *Mesospora* (generally less than 30 cells). Comments concerning the relationship between *Basispora* and *Mesospora*, which I regard as closely similar to one another, apart from their number of chloroplasts per cell, are reserved pending the sequence data of the generitype of *Basispora*. It is evident that further investigation is necessary to clarify the taxonomic status of the three genera considered here due to the inconsistencies of characters observed by different authors. This study nonetheless provided a basis for a reinstatement of the genus *Mesospora*, which are clearly distinguished from *Hapalospongidion* on morpho-anatomical and molecular grounds.

5.2.2 Interspecific morpho-anatomical distinction

5.2.2.1 *Mesospora* spp.

Specimens in their respective clade shared the same set of morpho-anatomical characters and are distinguished from other known species of *Mesospora* by a combination of characters as shown in Table 5.3. The species-level taxonomy of *Mesospora* is rather complicated because of morphological convergence and lack of reliable diagnostic features. In addition, considerable anatomical variation occurs within species, for instance in *M. negrosensis* which correlated with its high intraspecific genetic variation. Although it is hard to determine whether both types of reproductive structures occur on separate thallus due to thalli confluence, this appears to be the case for *Mesospora* because the two types of reproductive structures occur on distinct erect filaments as seen in *M. schmidtii*, *M. elongata*, *M. negrosensis* and *Mesospora* sp. 2.

The type species, *M. schmidtii* is distinguished from other species of the genus on the basis of its thin basal layer (separating it from *M. vanbosseae*), number of cells of erect filaments and stalks (separating it from *M. elongata* and *M. pangoensis* var. *galapagensis*), number of sterile terminal cells (distinguished from *Mesospora* sp. C and *Mesospora* sp. 2), organisation of vegetative filaments (distinguished from *M. negrosensis* morphotype 2, *Mesospora* sp. 1 and *Mesospora* sp. 2), largely biseriate plurilocular reproductive structures (separating it from *Mesospora* sp. D and *M. negrosensis* morphotype 1) and position of unilocular reproductive structures (distinguished from *M. macrocarpa*, *M. negrosensis* and *Mesospora* sp. 1). Since Weber-van Bosse (1911, 1913) did not designate a holotype among her specimens from the six syntype localities, a lectotype was recently (Poong et al., 2013) designated for *M. schmidtii* in the form of a microslide (L0790155) deposited in the Nationaal

Herbarium Nederland (L). At the same time, an epitype (microslide PSM 12246-GB7A-1) was chosen for *M. schmidtii* because the exact type locality of the lectotype was unknown.

The newly described *Mesospora elongata* is delimited from other species on the basis of its large number of cells of the erect filaments (up to 30 cells); hence the specific epithet and its long stalks (up to ten cells) on which unilocular reproductive structures are borne. Further examination of *M. elongata* which was initially described with unilocular reproductive structures terminally inserted on stalks of up to 8 cells, basal-lateral to the surrounding filaments (Poong et al., 2013; 2014) revealed that the unilocular reproductive structures are not necessarily basal to the surrounding filaments but they were also found to be inserted in the middle of the erect filaments. Although this new observation is a character specific to *M. macrocarpa*, these two species can still be distinguished by the higher number of cells in the stalk and higher number of sterile cells terminating the plurilocular bodies in *M. elongata*. Furthermore, the two types of reproductive structures are found on separate thallus in *M. elongata*, with the erect filaments of the thallus bearing unilocular reproductive structures consisting of a smaller number of cells (11 to 16 cells). Although *Mesospora pangoensis* var. *galapagensis* was also described with a large number of cells (25 to 35) and long stalks of eight to 12 cells, plurilocular bodies which are common in *Mesospora* was not reported. Additionally, the description of this species from the Galapagos by Setchell and Gardner (1937) was brief and thus further comparisons could not be made. *Mesospora schmidtii* described by Tanaka and Chihara (1982) from Japan with 20 to 28 (up to 32) cells per erect filament and five to 12 cells per stalk are probably *M. elongata*.

West and Calumpong (1996) reported that plurilocular reproductive structures of *M. negrosensis* from the field appear slightly different from those in culture.

Plurilocular reproductive structures of fertile thalli from the field generally have two to eight uniseriate loculi terminated by two to four inflated, rounded and highly vacuolated sterile cells. In contrast, plurilocular reproductive structures of cultured thalli possess three to four uniseriate loculi and one sterile terminal cell. In both cases, biseriate plurilocular reproductive structures were occasionally seen in the specimens. The two forms of plurilocular reproductive structures were observed in our field-collected specimens (Figs. 4.11e and g). Unilocular reproductive structures were not reported by West and Calumpong (1996), however they were present, albeit not fully matured, in some our specimens (Fig. 4.11h). They are inserted between the laterally cohesive lower filaments and laterally free upper filaments; the latter easily passes off as paraphyses. A hypothesis for the two morphotypes of *M. negrosensis* observed is, a special case of speciation-in-process, where the morphological distinctiveness has built up much faster than has the molecular one compared to the normal case where one expect to see diagnostic difference between newly evolved species after cessation of gene flow (Tautz et al., 2003). Another possible explanation is they might represent different strains of the same species. Nonetheless, it is of no doubt that the two morphotypes are authentically *M. negrosensis* since they form a strongly supported monophyletic clade with the gene sequence of the culture strain in all phylogenetic analyses (except for *cox*1-5' ML analysis).

Specimens of *Mesospora* sp. C collected in this study somehow differ from the description of the species by Lim et al. (2007) in their plurilocular reproductive structures. Current specimens of *Mesospora* sp. C were terminated by a single sterile cell but the original description indicated three sterile cells (comparison was made based on literature as the original specimen was not available for this study). Furthermore, unilocular reproductive structures of this species have been reported with three-celled stalks but this was not seen in the current batch of specimens. The plurilocular bodies of

this species are notably the shortest among currently examined species of *Mesospora*. As for *Mesospora* sp. D, plurilocular reproductive structures of specimens in this study agree with the description by Lim et al. (2007). While this species has been reported with unilocular reproductive structures borne on five-celled stalks, these structures were not seen in my specimens. Although unilocular reproductive structures are generally less common than plurilocular bodies in species of *Mesospora*, an increase of sample size may lead to collection of *Mesospora* spp. C and D with unilocular reproductive structures.

Mesospora sp. 1 is not easily distinguished from the type species at a mature stage, the notable difference lies in the distinctly enlarged sterile terminal cells of *Mesospora* sp. 1. However, the two are more easily separated when young as the basal portion of the erect filaments are more laterally cohesive in the former while the latter possess laterally free filaments in almost its entire length (except near the basal portion). In this context, *Mesospora* sp. 1 and *Mesospora* sp. D are very similar in the vegetative morphology of their young thallus, with the difference being the ratio of the laterally free to laterally cohesive parts of the erect filaments in the two species (see section 4.3.1.6).

Mesospora sp. 2 is unique among species of *Mesospora* because its plurilocular bodies-bearing filaments (see Fig. 4.16e) bear a resemblance to the demarcation of cortical and medullary layers in *Neoralfsia expansa*. However, this is not clearly seen if the basal layer is detached during the squashing process. The unilocular reproductive structures of this species are borne between the laterally free upper filaments and laterally cohesive lower filaments, similar to those in *M. negrosensis* and *Mesospora* sp. 1.

Regarding the three species of *Mesospora* not available for this study, the distribution of *M. macrocarpa* has so far been limited to the Mediterranean. In his

description of *M. mediterranea*, Feldmann (1937) noted that *Ralfsia macrocarpa* Feldmann which he previously described from Algeria (Feldmann, 1931) was in fact specimens of *M. mediterranea* overgrowing on *Ralfsia verrucosa*. Den Hartog (1968) also pointed out the conspecificity of *M. mediterranea* with *R. macrocarpa* and combined the two as *Mesospora macrocarpa* (Feldmann) den Hartog. In more recent years, Tanaka and Chihara (1982) in agreement with den Hartog (1968) regarded *M. macrocarpa* as a synonym of *M. mediterranea*. The two other species namely *M. vanbosseae* and *M. pangoensis* are distributed in the south Pacific. Børgesen (1924) distinguished *M. vanbosseae* from the type species based on its thick basal layer (separating it from other species of *Mesospora*), larger number of cells of the erect filaments which are cylindrical and thin walled, unregularly divided plurilocular bodies and absence of unilocular sporangia. Nevertheless, the characteristically thick basal layer was not clearly illustrated in Børgesen's figure (see Fig. 2.11).

Tanaka and Chihara (1982) transferred *Ralfsia pangoensis* Setchell and its variety to *Mesospora* on the basis of its similarity in vegetative and reproductive features to *M. schmidtii* rather than the Ralfsiaceae. According to Setchell (1924), *M. pangoensis* (as *Ralfsia pangoensis*) agree very closely to *M. schmidtii* in structure but is distinguished from the latter by unilocular sporangia which are terminal on its own stalk. Since Weber-van Bosse (1911, 1913) did not provide an illustration of the unilocular sporangia, Setchell (1924) interpreted them as sessile and lateral at the base of erect filaments. In absence of contrary statement, the unilocular reproductive structures observed by Weber-van Bosse are inserted on a stalk as the author wrote "*Les sporanges uniloculaires naissent latéralement à la base des filaments; M. Kuckuck en a observé, qui occupaient plus tard une position apicale par avortement du filament assimilateur; dans mes préparations ce filament avait persisté et le sporange etait inséré latéralement sur la cellule fertile"* which is translated as "Unilocular sporangia

arise laterally at the base of filaments; Mr Kuckuck has observed it to be occupying an apical position after abortion of the assimilatory filament, in my preparations this filament persisted and the sporangium was laterally inserted on the fertile cell". Coincidentally, only plurilocular reproductive structures were seen on the lectotype. It would not come as a surprise if sequence data of authentic specimens of *M. pangoensis* correspond to *M. schmidtii* or even *M. elongata*.

The generic and familial diagnoses of *Mesospora* and Mesosporaceae, respectively which were based on the type species seemed to require an amendment following the results of this study and a review of previous literatures. The erect filaments in species of *Mesospora* are 1) not necessarily mutually free in their whole length as seen in *M. negrosensis* and *Mesospora* sp. 2; and 2) the unilocular reproductive structures are not necessarily inserted lateral to the base of erect filaments as demonstrated in *M. macrocarpa*, *M. elongata*, *M. negrosensis, Mesospora* sp. 1 and *Mesospora* sp. 2.

Table 5.3 Morpho-anatomical comparison among species of *Mesospora*

Characters	*M. schmidtii* (Figs. 2.11, 4.10)	*M. vanbosseae* (Fig. 2.11)	*M. mesocarpa* (Fig. 2.11)	*M. pangoensis* (Fig. 2.11)	*M. pangoensis* var. *gala-pagensis*	*M. negrosensis* (Fig. 4.11)	*M. elongata* (Fig. 4.12)	*Mesospora* sp. C (Fig. 4.13)	*Mesospora* sp. D (Fig. 4.14)	*Mesospora* sp. 1 (Fig. 4.15)	*Mesospora* sp. 2 (Fig. 4.16)
Basal layer											
i.Thickness (µm)	ni	ni	ni	ni	ni	Up to 35	ni	ca. 7 to 15	ni	ca.16 to 26	ca. 10 to 12
ii.Number of cell layer(s)	1 to 4, usually 2	10 or more	Usually bilayer	2 to 3	ni	2 to 4	mostly 2	2 to 3	2 to 3	2 to 3	not determined
iii.Cell size and shape	Cells wider than high	7 µm long, ca. 18 µm wide	10 -15 µm wide	ni	ni	6 - 8 µm long, 11 – 13 µm wide	Cells wider than high	2.7 - 4.5 µm long, 2-4 times as broad	3.9-13.2µm long, 6.4-23.4 µm broad	7.3-25.1 µm wide, 3.4-10.9 µm long	3 to 4 times wider than long
Erect filaments	Distinct between unilocular & pluri-locular bearing filaments			Simple, densely packed		Two morphotypes	Distinct between unilocular & pluri-locular bearing filaments	Straight and free living	Straight and free living (mature)		Distinct between unilocular & pluri-locular bearing filaments
i.Thickness /length (µm)	ni	ni	550	200	ni	Usually less than 200	ni	127-187,	180-200	Up to 220	not determined
ii. Number of cell(s)	8 - 20	20 - 30	20 -25	18 - 20	25-35	Up to 20	11 - 29	8 to 17	8 to 17	6 to 18	not determined

Table 5.3 (continued)

Characters	*M. schmidtii* (Figs. 2.11, 4.10)	*M. vanbosseae* (Fig. 2.11)	*M. mesocarpa* (Fig. 2.11)	*M. pangoensis* (Fig. 2.11)	*M. pangoensis* var. *gala-pagensis*	*M. negrosensis* (Fig. 4.11)	*M. elongata* (Fig. 4.12)	*Mesospora* sp. C (Fig. 4.13)	*Mesospora* sp. D (Fig. 4.14)	*Mesospora* sp. 1 (Fig. 4.15)	*Mesospora* sp. 2 (Fig. 4.16)
iii. Cell size and shape	Lower cells: 6.7 - 14.8 (-17.7) µm long, 2.8 - 6.8 (-10.0) µm broad; length to diameter ratio was 1.2:1 to 3.5:1.Upper cells: 5.2 - 10.7 (-13.2) µm long, 4.3-8.8 (-11.2) µm broad; length to diameter ratio was 0.6:1 to 2.5:1	Cylindrical near the base; higher up the cells mostly broader than long. Up to 24 µm long and 8 -11 µm wide at the base and 16 µm in diameter near the top. Thin walls	10 -12 µm wide, 12 – 15 µm long; gradually wider up to 15 µm near the apex. Subcylin-drical, with rather thick membrane.	Apical cells are slightly thickened. Up to 8 µm in diameter.	Cylindrical. Apical cell pyriform to subglobose. 5 -7 µm in diameter; 1-2 times as long as broad.	11 – 15 µm long, 8 – 10 µm wide.	Basal cells: 6.3-15.9 (-23.7) µm long, 2.5-6.0 (-9.2) µm broad Distal cells:5.8-10.7 (-17.1) µm long, 3.4-7.9 (-13.2) µm broad	3.7 – 9.5 µm broad, 5.8 – 19.8 µm long, with a length to diameter ratio of 1:1 to 2.5:1	3.8 – 9.9 µm broad, 5.9 – 14.0 µm long with a length to diameter ratio of 0.7:1 to 2.5:1	3.2 – 12.2 µm broad and 5.7 - 20.4 µm long, with the length to diameter ratio of 0.6:1 to 3.5:1	Pluriloc. bearing filaments-Central: 4.7 – 8.4 µm wide, 5.3 – 13.6 µm long; Bottom:6.1 – 11.7 µm wide,10.9 – 31.2 µm long. Uniloc. bearing fil.- Upper half:4 – 8.3 µm wide, 5.1 – 15.5 µm long; lower half: 4.2 – 9.4 µm in diameter, 5.7 – 23.7 µm long

Table 5.3 (continued)

Characters	*M. schmidtii* (Figs. 2.11, 4.10)	*M. vanbosseae* (Fig. 2.11)	*M. mesocarpa* (Fig. 2.11)	*M. pangoensis* (Fig. 2.11)	*M. pangoensis* var. *gala-pagensis*	*M. negrosensis* (Fig. 4.11)	*M. elongata* (Fig. 4.12)	*Mesospora* sp. C (Fig. 4.13)	*Mesospora* sp. D (Fig. 4.14)	*Mesospora* sp. 1 (Fig. 4.15)	*Mesospora* sp. 2 (Fig. 4.16)
Unilocular rep. structure		Not observed			Scattered among the erect filaments, not in sori	First observed in this study	Only found in one specimen	Not observed in this study	Not observed in this study	Only found in one specimen	
i. length (μm)	24.2 - 143.2		70 – 100	100 – 120	115 – 125	15.9 - 99.6	25.9 - 76.7	ni	ni	37.1 – 70.7	29.9 – 88.3
ii. diameter (μm)	14.7- 54.8		45 – 60	60 -100	28 – 34	7.3 - 54.5	11.5 - 26.9	ni	ni	18.8 – 37.4	9.1 – 33.8
iii. no. of stalk cell(s)	Up to 4		1 to 2, sometimes sessile.	3 to 5	8 to 12	1 to 2, sometimes sessile	Up to 10	3	5	1 to 2	1 to 3
iv. Position	Lateral-basal to the erect filaments		Lateral at the base or usually in the middle of erect filaments.	Not lateral but terminal, each on its own stalk	ni	Terminally inserted on stalk cells, lateral and in the middle of the erect filaments	Terminal on stalk cells, lateral-basal or lateral-middle to the erect filaments	Terminal (position)	Terminal (position)	Terminally borne on stalk cells lateral to and in the middle of the erect filaments	Terminally inserted on stalk cells, lateral and in the middle of the erect filaments
v. Para-physis	absent		ni	ni	ni	absent	absent	absent	absent	absent	absent

Table 5.3 (continued)

Characters	*M. schmidtii* (Figs. 2.11, 4.10)	*M. vanbosseae* (Fig. 2.11)	*M. mesocarpa* (Fig. 2.11)	*M. pangoensis* (Fig. 2.11)	*M. pangoensis* var. *gala-pagensis*	*M. negrosensis* (Fig. 4.11)	*M. elongata* (Fig. 4.12)	*Mesospora* sp. C (Fig. 4.13)	*Mesospora* sp. D (Fig. 4.14)	*Mesospora* sp. 1 (Fig. 4.15)	*Mesospora* sp. 2 (Fig. 4.16)
Pluri-locular rep. structure	Intercalary with 8 locules on third and fourth cells from the apex.	Unsure, but not as regularly divided as in *M. schmidtii*. Formed at the upper part of erect filaments.	Subtermi-nal. Several irregular locules much less-regularly arranged than *M. schmidtii*.	Not observed	Not observed	Morphotype 1: uniseriate Morphotype 2: initially uniseriate, later biseriate	First uniseriate, later biseriate	First uniseriate and later biseriate	Mostly uniseriate, present in only one specimen	First uniseriate and later biseriate, present in only one specimen	First uniseriate and later biseriate
i. length (µm)	12.4 - 52.6	ni	ni			Morphotype 1: 11.6 – 17.5 Morphotype 2: 18.3 - 62.1	(13.8) 22.1 - 49.9	5.7 – 10.8 (this study)	12.9 - 42.5	27.5 – 59.2	14.9 – 58.1
ii. diameter (µm)	6.7 - 14.1	ni	ni			Morphotype 1: 4.5 – 5.0 Morphotype 2: 6.9 - 10.2	(4.8) 8.1 - 10.9	13.3 – 26.9 (this study)	6.4 - 7.4	7.7 – 12.8	4.8 – 12.0
iii. number of sterile terminal cell (s)	2	ni. 1 to 3 in the illustration	1?			Morphotype 1: 2 – 4 (enlarged) Morphotype 2: 1	2 - 4	3 (Lim et al. 2007), 1 (this study)	2 to 3	2 to 3	1

Table 5.3 (continued)

Characters	*M. schmidtii* (Figs. 2.11, 4.10)	*M. vanbosseae* (Fig. 2.11)	*M. mesocarpa* (Fig. 2.11)	*M. pangoensis* (Fig. 2.11)	*M. pangoensis* var. *gala-pagensis*	*M. negrosensis* (Fig. 4.11)	*M. elongata* (Fig. 4.12)	*Mesospora* sp. C (Fig. 4.13)	*Mesospora* sp. D (Fig. 4.14)	*Mesospora* sp. 1 (Fig. 4.15)	*Mesospora* sp. 2 (Fig. 4.16)
Additional remarks	Both types of rep. structure are found on separate thallus. Likely a single chloroplast per cell	An irregularly-lobed and bent plate-like plastid in which a single or two pyrenoid-like refractive bodies are present.	Each cell consists of a single plastid and many physodes.			One parietal, multi-lobed chloroplast per cell.First morphotype is laterally free through-out, the other is free in the upper portion and laterally cohesive in the lower portion	Both types of rep. structures are found on separate thallus. Likely a single chloroplast per cell	Single chloroplast ;erect filaments somewhat closely packed and not as readily separated as in *M. schmidtii* and *M. elongata*	Single chloroplast; erect filaments more closely packed in the young thallus; mature erect filaments were laterally free throughout	Likely a single chloroplast per cell	Likely a single chloroplast per cell. A three-layered structure in thalli bearing pluri-locular bodies
Type locality	Indonesia	Easter Island	Mediterra-nean Sea	Pago Pago Harbour, Tutuila Is., American Samoa	Conway Bay,Indefa-tigable Is., Galapagos	LaLaan, San Jose, Negros Oriental, Philippines	Fusaki, Ishigaki Island, Japan	Tg. Gemoh, Johor, Malaysia	Nakura, Ishigaki Island, Japan	Lendang Luar, Lombok Island	Labuhan Pandan, Lombok Island
References	Weber-van Bosse(1911, pp. 27-28; 1913, pp. 143-145); Poong et al. 2013; this study	Børgesen (1924, pp. 258-260).	Feldmann (1931, pp. 211-213; 1935, pp. 364-365; 1937, pp. 263-267)	Setchell (1924, pp. 167-168).	Setchell and Gardner (1937, pp. 74).	West and Calumpong (1996); Poong et al. (2014); this study	Poong et al. (2013; 2014);this study	Lim et al. (2007);this study	Lim et al. (2007); this study	This study	This study

5.2.2.2 *Diplura* spp.

Four genetic species of *Diplura*, tentatively designated as *Diplura* spp. 1 - 4 were identified in this study. In spite of their substantial genetic variation, they are barely distinguishable in morpho-anatomy. The 5'-end of *rbc*L gene in *Diplura* sp. 2 even requires its own set of primers for PCR amplification. As mentioned earlier in section 5.1.1, the significantly large genetic distance between *Diplura* spp. 1, 2 and 4, and *Diplura* sp. 3 suggest the latter to be of a separate but closely related genus, or the first three may simply be a single species with *Diplura* sp. 3 as a separate species. Nevertheless, this was not reflected in their anatomy as no outstanding anatomical differences were detected. Unilocular reproductive structures were seen in *Diplura* spp. 2, 3 and 4 while plurilocular bodies were present in all four species. Most of the time, a single sporangium is seen at the base of a paraphysis but insertion of more than one sporangium can be observed at times. Hollenberg (1969) and Tanaka and Chihara (1981b) did not observe unilocular reproductive structures for *D. simulans* and *D. simplex*, respectively and these structures were first mentioned in *Diplura* by Lim et al. (2007) for *D. simplex*.

The vegetative thallus of *Diplura* is simple in structure and yet it is difficult to obtain a proper slide preparation of the thallus which tends to break into uneven clumps upon squashing. Cell dimensions of the vegetative filaments in the Indo-Malay specimens are comparable to those reported for *D. simulans* and *D. simplex*. However, the size of their plurilocular reproductive structures are much shorter (less than 40 µm long) than those of *D. simulans* (up to 80 µm long) and *D. simplex* (up to 60 µm long). Apart from that, the plurilocular bodies of Indo-Malay specimens are biseriate and the two rows of loculi shared a single sterile terminal cell unlike those described in other species of *Diplura*. This in addition to the large genetic variation between Indo-Malay

and Japanese specimens further supports the recognition of the former as separate species. Despite Hollenberg's (1969) description of the plurangia of the type species being "mostly uniseriate in terminal pairs", the illustration given (see Fig. 2.14) showed a mixture of a) biseriate loculi on a single reproductive filament sharing a single sterile terminal cell, b) uniseriate loculi on a single reproductive filament terminated by a sterile cell and c) pairs of uniseriate loculi on a single reproductive filament, each row terminated by its own single sterile cell.

Tanaka and Chihara (1981b) proposed and distinguished *D. simplex* from *D. simulans* on the basis of the size and thickness of the crusts (thick and large crust in *D. simulans*; small and thin crust in *D. simplex*) and structure of erect filaments (branched, somewhat upwardly curved and readily separated in *D. simulans*; simple, straight and not as readily separated, in *D. simplex*). These features seemed insufficient for species delineation, and sequence data of *D. simulans* from the type locality is necessary for confirmation. Buchanan (2005) distinguished *Diplura* sp. "australis" from *D. simulans* and *D. simplex* based on its upright and strongly laterally adherent filaments, lack of hair pits and in being less gelatinous. Hair pits were reported in *D. simplex* but not in *D. simulans*, this character appears to be variable. In my opinion, these three probably represent a single species with slight variations. Two other undescribed species of *Diplura* from Japan, i.e. *Diplura* sp. B and *Diplura* sp. C were reported with morpho-anatomical features similar to *D. simplex*. The low interspecific variation among *D. simplex* and these two species [*p* distance: 3.36 – 5.27% (*rbc*L); 0.15% (*cox*1)] suggest the three to be conspecific.

Table 5.4 Morpho-anatomical comparison among species of *Diplura*

Species	Organisation of vegetative filaments	Plurilocular reproductive structures	Unilocular reproductive structures	References
D. simulans (Fig. 2.14)	Loosely held together by gelatinous matrix and readily separating under pressure	Mostly uniseriate in terminal pairs with a sterile terminal cell at the apex of each row of locules; 30-55 (-80) µm in length and 5 – 8.5 µm in diameter	Unknown and probably lacking	Hollenberg (1969)
D. simplex (Fig. 2.14)	Tightly adherent, not so readily separated	Standing in two rows on each erect filament, reproductive filament uni- or biseriate, bearing one sterile terminal cell; up to 60 µm long	Terminally inserted on one to two stalk cells. Paraphyses are present	Tanaka & Chihara (1981b), Lim et al. (2007)
Diplura sp. B	Tightly adherent	Mostly uniseriate, terminated by a single sterile terminal cell	Unknown	Lim et al. (2007)
Diplura sp. C	Tightly adherent	Mostly uniseriate, terminated by a single sterile terminal cell	Unknown	Lim et al. (2007)
Diplura sp. "australis"	Laterally coherent, separate only with considerable pressure	Usually uniseriate and in pairs, each reproductive filament has a single pale coloured sterile terminal cell; 25- 50 µm long and 5 - 7.5 µm broad	Unknown	Buchanan (2005)
Diplura sp. 1	Rather tightly adjoined	Initially uniseriate later biseriate, both rows of loculi shared a sterile terminal cell; 15.5 – 28.4 µm in length and 6.8 – 11.6 µm in diameter	Not observed	This study
Diplura sp. 2	Rather tightly adjoined	Initially uniseriate later biseriate, both rows of loculi shared a sterile terminal cell; 17.2 - 34.6 µm long and 7.1 – 10.6 µm wide	Terminally inserted on a single-celled stalk, basal-lateral to the paraphysis and terminal on the vegetative filaments ; 6.5 – 15.8 µm wide and 17.2 – 46.1 µm long	This study
Diplura sp. 3	Rather tightly adjoined	Initially uniseriate later biseriate, both rows of loculi shared a sterile terminal cell; 17.9 – 40.1 µm long and 6.1 – 11.6 µm wide	Terminally inserted on one to two stalk cells, basal-lateral to the paraphyses and terminal to the erect filaments; 6.5 – 12.2 µm wide and 19.1 – 27.8 µm long.	This study
Diplura sp. 4	Rather tightly adjoined	Initially uniseriate later biseriate, both rows of loculi shared a sterile terminal cell; 9.6 – 24.4 µm in length and 7.5 – 9.8 µm in diameter	Sessile, or terminally inserted on a stalk cell, terminal on the erect filaments, and lateral-basal to the paraphyses; 6.3 – 11.0 µm in diameter and 16.3 – 28.3 µm in length	This study

5.3 Overview of the biogeographic distribution of the crustose brown algae in this study

The present study reveals that the common crustose brown algae in Malaysia and Lombok Island are species of *Mesospora*, *Diplura* and *Neoralfsia expansa*. Despite global reports of crustose brown algae from the northern to southern hemisphere (e.g., Jaasund, 1965; Fletcher, 1987; Womersley, 1987), the distribution of certain genera or even species, are probably restricted to the cold ocean waters in the temperate, subpolar or subtropical regions. Members of the Ralfsiaceae (e.g., *R. fungiformis*, *A. japonicus* and *H. saxicola*) which have been reported mostly from the temperate or cold water region were not encountered in this study. The sole putative species of *Ralfsia* in the study (*Ralfsia* sp. 1) was collected from North America. Species of *Mesospora* are the more common brown crusts found in this region surrounded by the warm waters of the eastern Indian Ocean and the South China Sea (Dawson, 1954; Krishnamurthy and Baluswami, 1986; West and Calumpong, 1996; Sophiammal Nettar and Panikkar, 2009). In contrast, Japan and Hong Kong recorded a high diversity of crustose brown algal taxa (Kaehler, 1994; Kaehler, 1998; Tanaka and Chihara, 1980a-c; Tanaka and Chihara, 1981a-c; Tanaka and Chihara, 1982) presumably due to the influence of the Pacific Ocean and the outcome of a study dedicated to this group of algae.

Neoralfsia expansa has a cosmopolitan distribution and was frequently reported in the tropics and subtropics of both hemispheres (e.g., Ramírez and Santelices, 1991; Ribera et al., 1992; Rull Lluch, 2002; León-Alvarez and González-González, 2003; Lim et al., 2007; Kraft, 2009). As indicated by Kraft (2009), one reliable indicator of *N. expansa* as opposed to true *Ralfsia*; apart from the difference in the number of sterile cells terminating the plurilocular bodies (one in *N. expansa*, one to several in Ralfsiaceae), the number of chloroplasts per cell (one in *N. expansa*, one to several in

Ralfsiaceae) and the number of stalk cells (three to six stalk cells in *N. expansa*, sessile or one-celled stalk in Ralfsiaceae); is the place of collection as very few other ralfsioid algae occur regularly in the tropics.

The diversity of crustose brown algal species in Malaysia and Lombok Island were comparable, with some species being found in only one of the two places. *Mesospora schmidtii* was identified as the most prevalent crustose brown alga in Malaysia (Table 4.2), but this was not the case for Lombok Island. A possible explanation for this would be the frequency of collection. A single-trip collection from seven localities was made from Lombok Island whereas repeated trips were made for some of the same-locality collections in Malaysia. This study marks the first report of *M. negrosensis* outside the Philippines (West and Calumpong, 1996; Poong et al., 2014). DNA sequencing of *rbc*L and partial *cox*1 genes from Malaysian specimens matched those of the culture strain of *M. negrosensis* deposited in KU-MACC. The presence of *M. elongata* in Malaysia (east of Sabah) was discovered after the earlier report of this species in Lombok Island and Japan (Poong et al., 2013). However, the sole specimen was found in confluence with several other species of *Mesospora* and its existence was only evident from molecular data. The restricted geographical distribution of this species may likely be a result of limited sampling. Greater sampling effort across the coast of Sabah may potentially extend the distribution of this species in Malaysia. This also applies to the other crustose brown algal species, such as *Mesospora* sp. D which was previously recorded from Japan (Lim et al., 2007).

Apart from that, the presence of *Diplura* spp. in Malaysia and Indonesia is also documented for the first time here, expanding the known distribution range for this genus. Other members of this genus i.e. *D. simulans* and *D. simplex* were previously reported in the Pacific Coast of North America (Hollenberg, 1969; Pedroche, 2008), Japan (Tanaka and Chihara, 1981b; Lim et al., 2007) and Hong Kong (Kaehler, 1994),

while an unidentified species, *Diplura* sp. "australis" was recorded in New Zealand (Buchanan, 2005). The distribution of *Diplura* appears to be restricted to the Pacific.

The two species of *Stragularia* reported by Weber-van Bosse (1913) from Indonesia are potentially species of a different genus because members of the family Scytosiphonaceae are rarely if any, reported from warm water regions. The description (Weber-van Bosse, 1913) given for *S. clavata* was too brief and incomplete for definite identification; whereas for *S. polycarpa*, the thallus construction and unilocular reproductive structures described and illustrated bears a slight resemblance to those of *Diplura* spp. 2, 3 and 4. However, the number of sporangia described for *S. polycarpa* was four to five sporangia on one or two stalk cells as opposed to one to two sporangia on a single stalk cell in the *Diplura* spp. Furthermore, plurilocular reproductive structures which are common in *Diplura* spp. were not observed in *S. polycarpa*. To my knowledge, there is no further mention of *S. polycarpa* in the literatures since the first report by Weber-van Bosse. Therefore, I refrain from making any conclusions on the taxonomic placement of the two taxa especially considering that they were initially reported from other locations in Indonesia (not from Lombok Island).

A higher diversity of crustose brown algae remains to be uncovered as the global warm-water region are among some of the least well known in the phycology field. As we widen the geographical boundary of specimen collection, the existing distribution range of the specimens identified in this study is expected to be similarly expanded and in addition, allows us to determine whether certain taxa such as *Mesospora* sp. 2 and *Diplura* sp. 2 are endemic to Lombok Island.

CHAPTER 6: CONCLUSION

6.1 General conclusion and appraisal of this study

The objectives set for this study are achieved in which seven species of *Mesospora*, four genetic species of *Diplura*, a putative new genus and two yet-to-be described species were uncovered in Malaysia and Lombok Island in addition to the previously described *Neoralfsia expansa*; thus providing an improved understanding of the diversity of crustose brown algal flora in this warm water region. The use of a combined morpho-anatomical and molecular approach enables taxonomists to determine reliable diagnostic features for identification of crustose brown algal species. Previously published data were combined with newly generated data, and phylogenetic analyses were conducted to place newly described species in an evolutionary context. This study also provided new sequence data for species that had not previously been analysed in a genetic context, for instance *M. schmidtii*. Outputs of this study are summarised as follows:

1) The common crustose brown algae found in Malaysia and Lombok Island, Indonesia are species of *Mesospora*, *Diplura* and the monotypic *Neoralfsia expansa*.

2) Cold water taxa (for e.g., species of *Ralfsia*, *Analipus*, *Petroderma*) and crustose stages of heteromorphic brown algae are not encountered in this study. Several cold water species were however reported from the western Pacific, particularly in Japan and Hong Kong.

3) The diversity of crustose brown algal flora in Malaysia and Lombok Island are comparable; however, some species were only found in either Malaysia or Lombok Island.

4) The crustose brown algae are polyphyletic and are distributed in several clades, with members of Ralfsiales in one clade, while species of *Diplura* and the USP1 were resolved either separately or together near the root of the trees. Establishment of a new family to accommodate species of *Diplura* as suggested by Lim et al. (2007) appears warranted.

5) Circumscription of Ralfsiales include the Ralfsiaceae, Mesosporaceae, Neoralfsiaceae and a possible new family to accommodate the putative new genus which is anatomically similar to *Ralfsia* but is distinguished by its thin thallus and strictly erect vegetative filaments.

6) The combined use of *rbc*L and *cox*1-5' markers is suitable for molecular taxonomy and phylogeny studies of crustose brown algae.

7) *Mesospora* and *Hapalospongidion* should be treated as distinct genera on the basis of morpho-anatomy and molecular phylogeny; *H. gelatinosum* and *H. saxigenum* are probably conspecific.

8) The diagnosis of *Mesospora* requires the following amendment: a) partially or completely laterally free erect filaments and b) unilocular reproductive structures inserted laterally at the base or in the middle of erect filaments, to accommodate several species of *Mesospora* identified in this study.

9) This study led to the description of a new species of *Mesospora* i.e. *M. elongata* (Poong et al., 2013); potential new records of species of *Diplura* and *Mesospora* sp. D for Malaysia and Indonesia, and a new record of *M. negrosensis* (Poong et al., 2014) for Malaysia.

The first and third null hypotheses are therefore rejected whereby a) not all morphological characters were equally reliable as diagnostic feature and c) phylogenies of different molecular genetic markers were not congruent and do not have similar levels of resolution. Meanwhile, the second null hypothesis is accepted as the identification based on molecular phylogenies was indeed coherent with morphological characters. The present study along with those of Buchanan (2005) and Lim et al. (2007) laid the groundwork for future research on crustose brown algae incorporating molecular genetic approaches.

6.2 Future research direction

More work is necessary on the crustose brown algae, in particular sampling of genera that were previously assigned to the Ralfsiales such as *Acrospongium*, *Symphyocarpus*, *Sorapion*, *Zeacarpa* and *Basispora* for molecular studies in order to clarify their ambiguous taxonomic position. Aside from that, gene sequencing also needs to be carried out at the species level, especially for large genera such as *Ralfsia* and *Pseudolithoderma*. I foresee that many of the previously described species would be distantly related to the Ralfsiales; species of the same genus to be conspecific and a number of taxa may actually represent the crustose stages of heteromorphic brown algae.

The discovery of more crustose brown algal species from this region is anticipated following the exposure to DNA sequencing, subsequently altering the makeup of the diversity of this under represented group of brown algae as it was previously known from morphological descriptions. Widening of the biogeographic coverage, especially from the Atlantic and Mediterranean will increase chances of uncovering more crustose brown algal taxa. A revision of the taxonomy and

classification of crustose brown algae is inevitable and would certainly prove interesting. To this end, the use of gene sequence data is vital for proper taxonomic assignment. As an example, Kain et al. (2010) highlighted the need to re-examine the taxonomy and biogeography of *R. verrucosa*, the most common crustose species in temperate to cold waters of the world due to their earlier misidentification of *Colpomenia bullosa* (Saunders) Yamada and a putative species of *Ralfsia* as *R. verrucosa*.

Sequencing of type specimens is highly appreciated but are often not possible due to reasons which include limited amount of material available (thus destructive sampling is not permitted), degradation of the DNA of types conserved in herbariums, condition of the types conserved (for e.g., in formalin or as microslides) and even if recollection is conducted at the type locality, the "authentic" materials may not be found due to the altered landscape of the location or the species becoming extinct or increasingly rare in the wild. The suggestion by Tautz et al. (2003) for experienced taxonomists to identify newly collected specimens that could be used for DNA extraction and subsequently designated as a neotype may be a feasible solution to overcome the aforementioned problems. The advice of the International Code of Nomenclature of algae, fungi and plants is to select specimens from the original type locality and that are in good agreement with the original description of the species.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Publications

1. Poong S.W., Lim P.E., Phang, S.M., Gerung, G.S. and Kawai, H. 2013. *Mesospora elongata* sp. nov. (Ralfsiales, Phaeophyceae), a new crustose brown algal species from the Indo-Pacific region. Phycologia 52: 74 – 81.

2. Poong S.W., Lim P.E., Phang S.M., Sunarpi H., West J.A. and Kawai H. 2014. A molecular-assisted floristic survey of crustose brown algae (Phaeophyceae) from Malaysia and Lombok Island, Indonesia based on *rbc*L and partial *cox*1 genes. Journal of Applied Phycology 26:1231- 1242.

Conference and seminar presentations

1. An insight into the diversity of crustose brown algae (Phaeophyceae) in Malaysia and Lombok Island, Indonesia at the 21st International Seaweed Symposium (ISS) held in Bali, Indonesia from 21st – 26th April 2013. Awarded the University of British Columbia Graduate Student Paper Awards (third prize).

2. *Molecular phylogeny of crustose brown algae (Phaeophyceae) from Malaysia inferred using rbc*L *sequences* at the 17th Biological Sciences Graduate Congress (BSGC) held in Chulalongkorn University, Bangkok, Thailand from 8th – 10th December 2012.

3. *Molecular phylogeny of Malaysian Ralfsiales (Phaeophyceae)* for the postgraduate seminar at the Institute of Biological Sciences, Faculty of Science, University of Malaya on the 17th August 2011

4. *Molecular phylogeny of crustose brown algae (Phaeophyceae) from Malaysia inferred using rbc*L *and partial cox1 sequences* at the High Impact Research (HIR) student's seminar held in University of Malaya on the 27th June 2013

Posters presented

1. *Morphology and molecular phylogeny of Malaysian crustose brown algae (Phaeophyceae)* at the South China Sea (SCS) Conference held at the Complex of Institute of Research Management and Monitoring (IPPP), University of Malaya from 21st to 24th October 2012. Awarded the best poster award.

Appendix A. List of specimens examined in this study with information on voucher number, collection sites, collector(s), date of collection, latitudes and longitudes, and field number.

Taxa	Voucher number	Location and collector(s)	Date of collection	Latitude and longitude	Field number	DNA sequences and GenBank accession numbers where applicable *rbc*L/*cox*1-5’
Mesospora schmidtii Weber-van Bosse	PSM 12173	Pulau Che Kamat, Johor PEL	29 May 2009	N01° 21.090’ E104°13.918’	CHE 3	# / # (JQ619998/JQ620006)
Mesospora schmidtii	PSM 12179	Pulau Merambong,Johor PEL & SWP	24 Aug 2009	N01°19.000’ E103°37.016’	MER 10	+ / +
Mesospora schmidtii	PSM 12180	P. Merambong, PEL & SWP	24 Aug 2009	N01°19.000’ E103°37.016’	MER 11	+ / +
Mesospora schmidtii	PSM 12182	P. Merambong, PEL & SWP	24 Aug 2009	N01°19.000’ E103°37.016’	MER 13	+ / +
Mesospora schmidtii	PSM 12185	P.Merambong, PEL & SWP	24 Aug 2009	N01°19.000’ E103°37.016’	MER 16	+ / +
Mesospora schmidtii	PSM 12186	P.Merambong, PEL & SWP	24 Aug 2009	N01°19.000’ E103°37.016’	MER 17	+ / +
Mesospora schmidtii	PSM 12187	P.Merambong, PEL & SWP	24 Aug 2009	N01°19.000’ E 103°37.016’	MER 18	# / #
Mesospora schmidtii	PSM 12191	P.Merambong, PEL & SWP	24 Aug 2009	N01°19.000’ E103°37.016’	MER 22	+ / +
Mesospora schmidtii	PSM 12192	P.Merambong, PEL & SWP	24 Aug 2009	N01°19.000’ E103°37.016’	MER 23	+ / +
Mesospora schmidtii	PSM 12194	P.Merambong, PEL & SWP	24 Aug 2009	N01°19.000’ E103°37.016’	MER 71	+ / +
Mesospora schmidtii	PSM 12197	P.Merambong, PEL & SWP	24 Aug 2009	N 01°19.000’ E 103°37.016’	MER 97	* / +
Mesospora schmidtii	PSM 12201	KM 16, Port Dickson PEL & SWP	16 Dec 2009	N02°25.833’ E101°51.533’	POR 30	* / +
Mesospora schmidtii	PSM 12202	KM 16, P.D. PEL & SWP	16 Dec 2009	N02°25.833’ E101°51.533’	POR 31	+ / +

Mesospora schmidtii	PSM 12203	KM 16, P. D. PEL & SWP	16 Dec 2009	N02°25.833' E101°51.533'	POR 32	# / # (JQ620000/JQ620008)
Mesospora schmidtii	PSM 12207	KM 16, P. D. PEL & SWP	16 Dec 2009	N 02°25.833' E 101°51.533'	POR 96	+ / +
Mesospora schmidtii	PSM 12211	Pelabuhan Tanjung Langsat, Johor PEL	27 May 2010	N01°26.600' E104°0.333'	PTL 35	# / #
Mesospora schmidtii	PSM 12235	Batukijok, Lombok Island PEL & SWP	10 June 2010	S08°44.510' E116°01.381'	BAT 43	+ / + (JQ620001/JQ620009)
Mesospora schmidtii	PSM 12244	Kampong Dandulit, Sandakan PEL	9 Nov 2010	N05° 59.694' E117°54.555'	DAN 77	+ / +
Mesospora schmidtii	PSM 12245	Kampong Dandulit PEL	9 Nov 2010	N05° 59.694' E117°54.555'	DAN 78	# / #
Mesospora schmidtii	PSM12246 _GB7A	Batukijok PEL	1 May 2006	S08°44.536' E116°01.370'	BAT 7A	# / # (JQ619999/JQ620007)
Mesospora schmidtii	PSM 12317	Telok Kalong, Terengganu SWP	17 Feb 2012	N04°14.361' E103°27.136'	TKA 109	# /# (KC847387/KC847366)
Mesospora schmidtii	PSM 12318	Telok Kalong SWP	17 Feb 2012	N04°14.361' E103°27.136'	TKA 110	+ / +
Mesospora schmidtii	PSM 12333 _127B	Teluk Sari, Johor SWP	13 Mac 2012	N02°37.448' E103°45.465'	TKS 127B	+ / +
Mesospora schmidtii	PSM 12335	Teluk Sari, Johor SWP	13 Mac 2012	N02°37.448' E103°45.465'	TKS 129	+ / +
Mesospora schmidtii	PSM 12336	Teluk Sari, Johor SWP	13 Mac 2012	N02°37.448' E103°45.465'	TKS 130	+ / +
Mesospora schmidtii	PSM 12337	Teluk Sari, Johor SWP	13 Mac 2012	N02°37.448' E103°45.465'	TKS 131	+ / #
Mesospora schmidtii	PSM 12339	Teluk Ramunia, Johor SWP	15 Mac 2012	N01° 22.262' E104°16.933'	TKR 133	+ / #

Mesospora schmidtii	PSM 12344 _138E	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 138E	+ / +
Mesospora schmidtii	PSM 12345 _139A	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 139A	+ / +
Mesospora schmidtii	PSM 12346_140C	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 140C	+ / +
Mesospora schmidtii	PSM 12348	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 142	+ / +
Mesospora schmidtii	PSM 12350 _144C	Site 11, Semporna, Sabah PEL & SWP	1 July 2012	N04°35.34' E118°33.54'	SEM 144C	+ / +
Mesospora schmidtii	PSM 12353	Site 11, Semporna PEL & SWP	1 July 2012	N04°35.34' E118°33.54'	SEM 147	# /# (KC847388/KC847367)
Mesospora schmidtii	PSM 12354	Site 13, Kunak, Sabah PEL & SWP	2 July 2012	N04°39.66' E118°16.92'	KUN 148	+ / +
Mesospora schmidtii	PSM 12355	Site 13, Kunak PEL & SWP	2 July 2012	N04°39.66' E118°16.92'	KUN 149	+ / +
Mesospora schmidtii	PSM 12357	Site 21, Semporna, Sabah SWP	4 July 2012	N04°35.7' E118°43.26'	SEM 151	+ / +
Mesospora schmidtii	PSM 12358 _152A	Site 21, Semporna SWP	4 July 2012	N04°35.7' E118°43.26'	SEM 152A	+ / +
Mesospora schmidtii	PSM 12360	Site 24, Semporna, Sabah SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 156	+ / +
Mesospora schmidtii	PSM 12362 _158A	Site 24, Semporna SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 158A	+ / +
Mesospora schmidtii	PSM 12364 _160A	Site 24, Semporna SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 160A	+ / +
Mesospora schmidtii	PSM 12365 _161A	Site 24, Semporna SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 161A	+ / +
Mesospora schmidtii	PSM 12366	Site 25, Semporna, Sabah SWP	6 July 2012	N04°18.9' E118°27.3'	SEM 162	+ / +

Mesospora schmidtii	PSM 12367	Site 25, Semporna SWP	6 July 2012	N04°18.9' E118°27.3'	SEM 163	+ / +
Mesospora schmidtii	PSM 12368	Site 25, Semporna SWP	6 July 2012	N04°18.9' E118°27.3'	SEM 164	+ / +
Mesospora schmidtii	PSM 12369	Site 25, Semporna SWP	6 July 2012	N04°18.9' E118°27.3'	SEM 165	+ / +
Mesospora schmidtii	PSM 12370	Site 25, Semporna SWP	6 July 2012	N04°18.9' E118°27.3'	SEM 166	+ / +
Mesospora schmidtii	PSM 12372	Telok Pelandok, Port Dickson SWP	30 July 2012	N02°25.134' E101°53.12'	POR 168	+ / #
Mesospora elongata Poong, Lim & Phang	PSM 12214	Nipah, Lombok Island PEL & SWP	8 June 2010	S08°26.146' E116°02.523'	NIP 66	# /# (JQ620005/JQ620013)
Mesospora elongata	PSM 12221	Gili Genting, Lombok Island PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 38	# / #
Mesospora elongata	PSM 12225	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 56	+ / + (JQ620004/JQ620012)
Mesospora elongata	PSM 12234	Nipah PEL & SWP	10 June 2010	S08°44.510' E116°01.381'	BAT 39	+ / +
Mesospora elongata	KU-d11205	Fusaki, Ishigaki Island, Okinawa Prefecture, Japan PEL	6 Feb 2005	N.A.	FUS 2	+ / + (JQ620002/JQ620010)
Mesospora elongata	KU-d11206	Fusaki PEL	6 Feb 2005	N.A.	FUS 3	# /# (JQ620003/JQ620011)
Mesospora elongata	PSM 12363 _159D	Site 24, Semporna SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 159D	# / #
Mesospora negrosensis West & Calumpong	KU 1065	-				# /# (KC847389/KC847368)
Mesospora negrosensis	PSM 12171	Pulau Che Kamat PEL & SWP	29 May 2009	N01° 21.090' E104°13.918'	CHE 1	+ / +
Mesospora negrosensis	PSM 12183	P. Merambong PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 14	# /# (KC847390/KC847369)

Mesospora negrosensis	PSM 12184	P. Merambong, PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 15	+ / +
Mesospora negrosensis	PSM 12249 _63	Bise, Motobu, Okinawa Prefecture, Japan PEL	10 July 2010	N.A.	BIS 63	+ / +
Mesospora negrosensis	PSM 12251	Bise, Motobu PEL	10 July 2010	N.A.	BIS 62	# / #
Mesospora negrosensis	PSM 12324 _117A	Pantai Chendering, Terengganu SW.P	16 Feb 2012	N05°16.096' E103°11.147'	CHD 117A	+ / +
Mesospora negrosensis	PSM 12326	P. Chendering SWP	16 Feb 2012	N05°16.096' E103°11.147'	CHD 120	# /# (KC847391/KC847370)
Mesospora negrosensis	PSM 12328 _122A	P.Chendering SWP	16 Feb 2012	N05°16.096' E103°11.147'	CHD 122A	+ / +
Mesospora negrosensis	PSM 12363 _159A	Site 24, Semporna SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 159A	# / #
Mesospora negrosensis	PSM 12365 _161B	Site 24, Semporna SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 161B	+ / +
Mesospora sp. C	PSM 12205	KM 16, P.D. PEL & SWP	16 Dec 2009	N02°25.833' E101°51.533'	POR 34	# / #
Mesospora sp. C	PSM 12333 _127A	Teluk Sari, Johor SWP	13 Mac 2012	N02°37.448' E103°45.465'	TKS 127A	# / #
Mesospora sp. C	PSM 12338	Teluk Ramunia SWP	15 Mac 2012	N01° 22.262' E104°16.933'	TKR 132	* / +
Mesospora sp. C	PSM 12344 _138C	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 138C	+ / +
Mesospora sp. C	PSM 12346 _140E	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 140E	* / +
Mesospora sp. C	PSM 12349 _143A	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 143A	# / #
Mesospora sp. D	KU-d11207	Nakura, Ishigaki Island,Okinawa Pref.,Japan PEL	6 Feb 2005	N.A.	NAK 4	# / *

Mesospora sp. D	PSM 12175	Pulau Che Kamat PEL	29 May 2009	N01° 21.090' E104°13.918'	CHE 5	+ / +
Mesospora sp. D	PSM 12188	P.Merambong PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 19	# / #
Mesospora sp. D	PSM 12220	Batu Layar, Lombok Island PEL & SWP	8 June 2010	S08°31.012' E116°03.64'	LAY 86	# / #
Mesospora sp. D	PSM 12346 _140F	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 140F	# / #
Mesospora sp. 1	PSM 12212	Lendang Luar, Lombok Island PEL & SWP	8 June 2010	S08°27.719' E116°02.164'	LEN 41	# / #
Mesospora sp. 1	PSM 12226 _57B	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 57B	+ / +
Mesospora sp. 1	PSM 12232	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 88	# / #
Mesospora sp. 1	PSM 12319	Pantai Kemasik, Terengganu SWP	17 Feb 2012	N04°24.940' E103°27.834'	KEM 111	# / #
Mesospora sp. 1	PSM 12320	Pantai Kemasik SWP	17 Feb 2012	N04°24.940' E103°27.834'	KEM 112	+ / +
Mesospora sp. 1	PSM 12321	Pantai Kemasik, SWP	17 Feb 2012	N04°24.940' E103°27.834'	KEM 113	+ / +
Mesospora sp. 1	PSM 12323	Pantai Kemasik SWP	17 Feb 2012	N04°24.940' E103°27.834'	KEM 115	+ / +
Mesospora sp. 1	PSM 12324 _117B	P.Chendering SWP	16 Feb 2012	N05°16.096' E103°11.147'	CHD 117B	# / #
Mesospora sp. 1	PSM 12328 _122B	P.Chendering SWP	16 Feb 2012	N05°16.096' E103°11.147'	CHD 122B	+ / +
Mesospora sp. 1	PSM 12346 _140D	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 140D	+ / +
Mesospora sp. 2	PSM 12218	Batu Layar, PEL & SWP	8 June 2010	S08°31.012' E116°03.64'	LAY 55	# / #
Mesospora sp. 2	PSM 12239	Labuhan Pandan, Lombok Island PEL & SWP	12 June 2010	S08°29.203' E116°39.695'	LAB 74	+ / +

Mesospora sp. 2	PSM 12240 _76	Labuhan Pandan PEL & SWP	12 June 2010	S 08°29.203' E 116°39.695'	LAB 76	+ / +
Mesospora sp. 2	PSM 12241 _79	Labuhan Pandan PEL & SWP	12 June 2010	S08°29.203' E116°39.695'	LAB 79	# / #
Mesospora sp. 2	PSM 12241 _81	Labuhan Pandan PEL & SWP	12 June 2010	S08°29.203' E116°39.695'	LAB 81	+ / +
Mesospora sp. 2	PSM 12242 _83	Labuhan Pandan PEL & SWP	12 June 2010	S 08°29.203' E 116°39.695'	LAB 83	+ / +
"*Mesospora*" sp. 3	PSM 12189	P.Merambong PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 20	# / #
"*Mesospora*" sp. 3	PSM 12223 _45	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 45	# / #
"*Mesospora*" sp. 3	PSM 12226 _57A	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 57A	+ / +
"*Mesospora*" sp. 3	PSM 12233	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 89	+ / +
"*Mesospora*" sp. 3	PSM 12236 _47B	Rambang, Lombok Island PEL & SWP	11 June 2010	S08°43.893' E116°33.103'	RAM 47B	# / #
"*Mesospora*" sp. 3	PSM 12238	Labuhan Pandan PEL & SWP	12 June 2010	S08°29.203' E116°39.695'	LAB 53	# / #
"*Mesospora*" sp. 3	PSM 12352	Site 11, Semporna PEL & SWP	1 July 2012	N 04°35.22' E 118°47.28'	SEM 146	# / #
"*Mesospora*" sp. 3	PSM 12358 _152E	Site 21, Semporna SWP	4 July 2012	N04°35.7' E118°43.26'	SEM 152E	+ / +
"*Mesospora*" sp. 3	PSM 12364 _160C	Site 24, Semporna SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 160C	+ / +
"*Mesospora*" sp. 3	PSM 12775_ 170	The island west of Alor Island, Indonesia S. Draisma	10 April 2013	S08°16.104' E124°24.179'	ALO 170	+ / *
Neoralfsia expansa (J.Agardh) Lim & Kawai ex Cormaci & G. Furnari	PSM 12213 _50B	Nipah PEL & SWP	8 June 2010	S08°26.146' E116°02.523'	NIP 50B	# / #
Neoralfsia expansa	PSM 12219	Batu Layar PEL & SWP	8 June 2010	S08°31.012' E116°03.64'	LAY 85	# / #

Neoralfsia expansa	PSM 12223 _44	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 44	+ / #
Neoralfsia expansa	PSM 12223 _72	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 72	# / -
Neoralfsia expansa	PSM 12230	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 70	+ /+ (KC847393/KC847372)
Neoralfsia expansa	PSM 12247	Katori, Chiba Prefecture, Japan PEL	5 Aug 2009		KAT 49	# / #
Neoralfsia expansa	PSM 12248	Bise, Motobu, PEL	10 July 2010		BIS 58	# / #
Neoralfsia expansa	PSM 12249 _73	Bise, Motobu, PEL	10 July 2010		BIS 73	+ / +
Neoralfsia expansa	PSM 12254	Pulau Besar, Melaka S. Draisma	11 Apr 2010	N02°7.000' E101°19.98'	BES 40	# /# (KC847392/KC847371)
Neoralfsia expansa	PSM 12322[1]	Pantai Kemasik SWP	17 Feb 2010	N04°24.940' E103°27.834'	KEM 114	# /# (KC847394/KC847373)
Neoralfsia expansa	PSM 12342	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 136	+ / #
Neoralfsia expansa	PSM 12775 _171	The island west of Alor Island, Indonesia S. Draisma	10 April 2013	S08°16.104' E124°24.179'	ALO 171	+ / +
Unidentified crustose brown algal species 1	PSM 12174	Pulau Che Kamat PEL	29 May 2009	N01° 21.090' E104°13.918'	CHE 4	# / #
Unidentified crustose brown algal species 1	PSM 12176	Pulau Che Kamat, PEL	29 May 2009	N01° 21.090' E104°13.918'	CHE 6	+ / +
Unidentified crustose brown algal species 1	PSM 12177	Pulau Che Kamat PEL	29 May 2009	N01° 21.090' E104°13.918'	CHE 7	+ / -
Unidentified crustose brown algal species 1	PSM 12190	P.Merambong PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 21	# / #
Unidentified crustose brown algal species 1	PSM 12195	P. Merambong PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 91	+ / +

Unidentified crustose brown algal species 1	PSM 12196	P.Merambong PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 95	+ / +
Unidentified crustose brown algal	PSM 12198	KM 16, P.D. PEL & SWP	16 Dec 2009	N02°25.833' E101°51.533'	POR 27	# / #
Unidentified crustose brown algal species 1	PSM 12316	Pantai Dickson SWP	8 Nov 2011	N02°25.037' E101°53.695'	POR 100	+ / #
Ralfsia sp. 1	PSM 12255	Plymouth, MA, US PEL	NA	NA	USA 36	+ / -
Ralfsia sp. 1	PSM 12256	Plymouth PEL	NA	NA	USA 37	# / #
Hapalospongidion saxigenum	ASL418	Ringaringa, Stewart Island, New Zealand W. Nelson	27 Nov 2011	NA	NEW 106	+ / -
Unidentified crustose brown algal species 2	PSM 12217	Batu Layar PEL & SWP	8 June 2010	S08°31.012' E116°03.64'	LAY 54	# / #
Diplura simplex Tanaka & Chihara	KU-d2582	Maruyama, Awaji Island, Hyogo Pref., Japan	12 May 2005	N.A.	MRY 121	+ / +
Diplura sp. B	KU-d2574	Maruyama, Awaji Island, Hyogo Pref., Japan	12 May 2005	N.A.	MRY 222	# / #
Diplura sp. 1	PSM 12178	P. Merambong PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 8	+ / -
Diplura sp. 1	PSM 12199	KM 16, P.D. PEL & SWP	16 Dec 2009	N02°25.833' E101°51.533'	POR 28	*/ -
Diplura sp. 1	PSM 12200	KM 16, P. D. PEL & SWP	16 Dec 2009	N02°25.833' E101°51.533'	POR 29	+ / -
Diplura sp. 1	PSM 12204	KM 16, P.D. PEL & SWP	16 Dec 2009	N02°25.833' E101°51.533'	POR 33	# / #
Diplura sp. 1	PSM 12206	KM 16, P.D. PEL & SWP	16 Dec 2009	N02°25.833' E101°51.533'	POR 94	+ / +

Diplura sp. 1	PSM 12208	Pantai Dickson PEL & SWP	16 Dec 2009	N02°25.037' E101°53.695'	POR 24	# /# (KC847395/KC847374)
Diplura sp. 1	PSM 12210	Pantai Dickson PEL & SWP	16 Dec 2009	N02°25.037' E101°53.695'	POR 26	+ / +
Diplura sp. 1	PSM 12222	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 42	# /# (KC847396/KC847375)
Diplura sp. 1	PSM 12325	P.Chendering SWP	16 Feb 2012	N05°16.096' E103°11.147'	CHD 118	# /# (KC847397/KC847376)
Diplura sp. 1	PSM 12327	P.Chendering SWP	16 Feb 2012	N05°16.096' E103°11.147'	CHD 121	+ / +
Diplura sp. 1	PSM 12329	P.Chendering SWP	16 Feb 2012	N05°16.096' E103°11.147'	CHD 123	+ / +
Diplura sp. 1	PSM 12332	Teluk Sari SWP	13 Mac 2012	N02°37.448' E103°45.465'	TKS 126	+ / -
Diplura sp. 1	PSM 12334	Teluk Sari SWP	13 Mac 2012	N02°37.448' E103°45.465'	TKS 128	# / #
Diplura sp. 1	PSM 12359	Site 24, Semporna SWP	5 July 2012	N04°35.22' E118°47.28'	SEM 155	# /# (KC847398/KC847377)
Diplura sp. 1	PSM 12373	Telok Pelandok, Port Dickson SWP	30 July 2012	N02°25.134' E101°53.12'	POR 169	+ / #
Diplura sp. 2	PSM 12236 _47A	Rambang PEL & SWP	11 June 2010	S08°43.893' E116°33.103'	RAM 47A	# / #
Diplura sp. 2	PSM 12314	Rambang, PEL & SWP	11 June 2010	S08°43.893' E116°33.103'	RAM 65	+ / +
Diplura sp. 2	PSM 12237	Labuhan Pandan PEL & SWP	12 June 2010	S08°29.203' E116°39.695'	LAB 46	# / #
Diplura sp. 2	PSM 12240 _75	Labuhan Pandan PEL & SWP	12 June 2010	S08°29.203' E116°39.695'	LAB 75	+ / +
Diplura sp. 2	PSM 12241 _80	Labuhan Pandan PEL & SWP	12 June 2010	S 08°29.203' E 116°39.695'	LAB 80	+ / +
Diplura sp. 2	PSM 12242 _82	Labuhan Pandan PEL & SWP	12 June 2010	S08°29.203' E116°39.695'	LAB 82	+ / +
Diplura sp. 2	PSM 12243	Labuhan Pandan PEL & SWP	12 June 2010	S08°29.203' E116°39.695'	LAB 84	+ / +

Diplura sp. 3	PSM 12172	Pulau Che Kamat PEL	29 May 2009	N01° 21.090' E104°13.918'	CHE 2	# /# (KC847399/KC847378)
Diplura sp. 3	PSM 12193	P.Merambong PEL & SWP	24 Aug 2009	N01°19.000' E103°37.016'	MER 64	# / #
Diplura sp. 3	PSM 12209	Pantai Dickson PEL & SWP	16 Dec 2009	N02°25.037' E101°53.695'	POR 25	* / +
Diplura sp. 3	PSM 12215	Batu Layar PEL & SWP	8 June 2010	S08°31.012' E116°03.64'	LAY 51	# /# (KC847401/KC847380)
Diplura sp. 3	PSM 12216	Batu Layar PEL & SWP	8 June 2010	S08°31.012' E116°03.64'	LAY 52	+ / +
Diplura sp. 3	PSM 12224 _48B	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 48B	# /# (KC847400/KC847379)
Diplura sp. 3	PSM 12227	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 67	+ / *
Diplura sp. 3	PSM 12229	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 69	*/ *
Diplura sp. 3	PSM 12231	Gili Genting PEL & SWP	10 June 2010	S08°43.834' E115°57.937'	GIL 87	* / -
Diplura sp. 3	PSM 12315	Pantai Dickson SWP	8 Nov 2011	N02°25.037' E101°53.695'	POR 99	+ / +
Diplura sp. 3	PSM 12350 _144E	Site 11, Semporna PEL & SWP	1 July 2012	N04°35.34' E118°33.54'	SEM 144E	# / #
Diplura sp. 3	PSM 12371	Pantai Dickson SWP	30 July 2012	N02°25.037' E101°53.695'	POR 167	# /# (KC847402/KC847381)
Diplura sp. 4	PSM 12340	Pulau Sayak, Kedah Y.Y. Yow	25 Mac 2012	N05°39.656' E100°20.191'	SAY 134	+ / +
Diplura sp. 4	PSM 12341	Pulau Sayak Y.Y. Yow	25 Mac 2012	N05°39.656' E100°20.191'	SAY 135	+ / +
Diplura sp. 4	PSM 12343	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 137	+ / +
Diplura sp. 4	PSM 12345 _139B	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 139B	+ / +
Diplura sp. 4	PSM 12347	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 141	# / #

Diplura sp. 4	PSM 12349 _143C	Pulau Che Kamat SWP	16 Mac 2012	N01° 21.090' E104°13.918'	CHE 143C	+ / +

GenBank accession numbers were provided for taxa of which DNA sequences were deposited in GenBank. Abbreviations for collectors are: PEL – Phaik Eem Lim; SWP – Sze Wan Poong; W. Nelson – Wendy A. Nelson; S. Draisma – Stefano G.A. Draisma; Y.Y. Yow – Yoon Yen Yow. PSM, KU and KU-d indicate reference code of vouchers at the University of Malaya Seaweeds and Seagrasses Herbarium (KLU), culture obtained from Kobe University Macroalgal Culture Collection (KU-MACC) and the herbarium of the Kobe University Research Center for Inland Seas respectively. All specimens were attached to rocks unless specified otherwise. Sequences obtained and included in phylogenetic analyses are denoted with '#'. Sequences obtained but not included in phylogenetic analyses are denoted with '+'. Sequences with ambiguities or incomplete sequence are denoted with '*'. Unavailable sequences are denoted with '-´.

[1]This specimen was found attached to shells.

Appendix B. Sources of published sequences used for phylogenetic analyses of the combined and individual data sets. A dash indicates unavailable data.

Taxa	Higher taxonomic rank (Class/Order/Family)	Collection site, date, voucher or reference of *rbc*L/*cox*1 sequences	Genbank accession number *rbc*L/ *cox*1
Ectocarpus siliculosus (Dillwyn) Lyngbye	Ectocarpales	Valentin and Zetsche, 1990/ Bittner et al., 2008	X52503/ EU579867
Scytosiphon lomentaria (Lyngbye) Link		Kogame et al,. 1999/ Silberfeld et al., 2010	AB022238/EU681424
Dictyosiphon foeniculaceus (Hudson) Greville		Siemer et al., 1998/ Silberfeld et al., 2011	AF055397/ JF796539
Asterocladon interjectum Uwai, Nagasato, Motomura & Kogame	Asterocladales	Uwai et al., 2005/-	AB102866/-
Asterocladon lobatum Müller, Parodi & Peters		Peters and Ramírez, 2001/-	AJ295824/-
Laminaria digitata (Hudson) Lamouroux	Laminariales	Cho et al., 2004/ Oudot-Le Secq et al., 2002	AY372984/AJ344328
Chorda filum (Linnaeus) Stackhouse		Kawai et al,. 2001/ Ehara et al. (Unpublished)	AB035786/AF037991
Padina gymnospora (Kützing) Sonder	Dictyotales	Bittner et al., 2008/ Bittner et al., 2008	EU579933/EU579871
Dictyota dichotoma (Hudson) J.V. Lamouroux		Draisma et al., 2001/ Oudot-Le Secq et al., 2006	AJ287852/AY500368
Ascophyllum nodusum (Linnaeus) Le Jolis	Fucales	Draisma et al., 2001/ Silberfeld et al., 2010	AJ287853/ EU681390
Sargassum muticum (Yendo) Fensholt		Draisma et al., 2001/ Silberfeld et al., 2010	AJ287854/ EU681423
Syringoderma phinneyi Henry & Müller	Syringodermatales	Draisma et al., 2001/ Silberfeld et al., 2010	AJ287868/ EU681429
Microzonia velutina (Harvey) J. Agardh		Burrowes et al,. 2003/-	AY157697/ -
Onslowia endophytica Searles	Onslowiales	Draisma et al., 2001/ Bittner et al., 2008	AJ287864/ EU579870
Verosphacella ebrachia E.C. Henry		Draisma et al., 2001/-	AJ287867/-
Sphacelaria cirrosa (Roth) C. Agardh	Sphacelariales	Draisma et al., 2001/-	AJ287865/-
Halopteris filicina (Grateloup) Kützing		Draisma et al. (unpublished) /Bittner et al., 2008	AJ287894/ EU579868
Phaeostrophion irregulare Setchell & Gardner	Phaeostrophiaceae	Kawai et al., 2005/-	AB117948/-
Scytothamnus australis (J.Agardh) Hooker & Harvey	Scytothamnales	Peters and Ramírez, 2001/ Silberfeld et al., 2010	AJ295833/ EU681425

Splachnidium rugosum (Linnaeus) Greville		Peters and Ramírez, 2001/ Silberfeld et al., 2010	AJ295834/ EU681427
Tribonema aequale Pascher	Xanthophyceae	Bailey and Andersen (unpublished)/Ehara et al., 1997	AF084611/AB000211
Phaeothamnion confervicola Lagerheim	Phaeothamniophyceae	Bailey et al., 1998/-	AF064746/ -
Schizocladia ischiensis Henry, Okuda & Kawai	Schizocladiophyceae	Kawai et al., 2003/-	AB085615/-
Choristocarpus tenellus (Kützing) Zanardini	Discosporangiales	Draisma et al., 2001/-	AJ287861/-
Discosporangium mesarthrocarpum (Meneghini) Hauck		Kawai et al., 2007/-	AB252654/-
Ishige sinicola (Setchell & Gardner) Chihara	Ishigeales	Cho et al., 2004/-	AY372976/-
Ishige okamurae Yendo		Kawai et al., 2005/ Bittner et al., 2008	AB117951/EU579869
Himantothallus grandifolius (A. & E. Gepp) Zinova	Desmarestiales	Silberfeld et al., 2010/ Silberfeld et al., 2010	GQ368320/GQ368262
Desmarestia tabacoides Okamura		Kawai and Sasaki, 2000/Yang et al. (unpublished)	AB037140/HE866763
Tilopteris mertensii (Turner) Kützing	Tilopteridales	Sasaki et al., 2001/ Silberfeld et al., 2010	AB045260/EU681430
Cutleria multifida (J.E. Smith) Greville		Burrowes et al., 2003/ Silberfeld et al., 2010	AY157692/EU681398
Phyllariopsis brevipes (C. Agardh) Henry & South		Sasaki et al., 2001/ Silberfeld et al., 2010	AB045244/GQ368264
Phaeosiphoniella cryophila Hoope, Henry & Kuhlenkamp	Phaeosiphoniellaceae	Phillips et al., 2008/-	EF990254/-
Nemoderma tingitanum Schousboe ex Bornet	Nemodermatales	Phillips et al., 2008/-	EF990253/-
Sporochnus pedunculatus (Hudson) C. Agardh	Sporochnales	Bittner et al., 2008/ Silberfeld et al., 2010	EU579937/EU681428
Bellotia eriophorum Harvey		Silberfeld et al., 2010/ Silberfeld et al., 2010	GQ368313/GQ368255
Ascoseira mirabilis Skottsber	Ascoseirales	Phillips et al., 2008/ Silberfeld et al., 2010	EF990237/ EU681391
Heribaudiella fluviatilis (Areschoug) Svedelius	*Incertae sedis*	Draima et al., 2010/-	FM956111/-
Pseudolithoderma roscoffense Loiseaux		Bittner et al., 2008/ Bittner et al., 2008	EU579935/EU579872

Petroderma maculiforme (Wollny) Kuckuck		Bittner et al., 2008/-	EU579934/-
Porterinema fluviatile (Porter) Waern		Draisma et al., 2010/-	FM956108/-
Diplura simplex Tanaka & Chihara		Lim et al., 2007/This study	AB250084/KC847385
Diplura sp. B		Lim et al., 2007/This study	AB250086/KC847386
Diplura sp. C		Lim et al., 2007/-	AB250087/-
Analipus japonicus (Harvey) Wynne	Ralfsiales	Lim et al., 2007/ Silberfeld et al., 2010	AB264042/EU681389
Heteroralfsia saxicola (Okamura& Yamada) Kawai		Lim et al., 2007/-	AB250070/ -
Ralfsia fungiformis (Gunnerus) Setchell & Gardner		Lim et al., 2007/ Bittner et al., 2008	AB250071/EU579873
Ralfsia sp. B		Lim et al., 2007/-	AB250074/-
Ralfsia sp. C		Lim et al., 2007/-	AB250075/-
Mesospora sp. C		Lim et al., 2007/ This study	AB250065/KC847382
Mesospora sp. D		Lim et al., 2007/-	AB250066/-
Mesospora sp. G		Lim et al., 2007/-	AB250069/-
Neoralfsia expansa (J.Agardh) Lim & Kawai ex Cormaci & G. Furnari		Lim et al., 2007/ Poong et al.,2014	AB250077/KC847383
Neoralfsia expansa		Lim et al., 2007/ Poong et al.,2014	AB250078/KC847384

Appendix C: Uncorrected pairwise distance matrix of the *rbc*L gene sequences

	Ajapon	R.fungi	H.saxicola	USA37	USA36	RalfspB	RalfspC	NexpDes	CHE136	KEM114	LAY85	BES40	BIS73	BIS58	KAT49	GIL72	GIL70	NIP50B	GIL44	ALO171	GIL45	GIL89	MER20	GIL57A
A.japon	N																							
R.fungi	0.05616	N																						
H.saxicola	0.07424	0.08243	N																					
USA37	0.08389	0.09675	0.10171	N																				
USA36	0.08395	0.0968	0.10175	0	N																			
RalfspB	0.08463	0.0975	0.10245	0.00074	0.00074	N																		
RalfspC	0.09668	0.10457	0.10886	0.03573	0.03574	0.03571	N																	
NexpDes	0.11029	0.11731	0.11927	0.11709	0.11713	0.11634	0.11388	N																
CHE136	0.10616	0.11388	0.11507	0.11284	0.11289	0.11359	0.11116	0.00375	N															
KEM114	0.1069	0.11692	0.11581	0.11507	0.11512	0.11581	0.11497	0.01201	0.00817	N														
LAY85	0.11118	0.11518	0.11718	0.11497	0.11502	0.11495	0.11426	0.01597	0.01293	0.0137	N													
BES40	0.10678	0.11373	0.11419	0.11346	0.11351	0.11343	0.11428	0.02034	0.01715	0.01641	0.01369	N												
BIS73	0.1069	0.1131	0.11359	0.11284	0.1129	0.11359	0.1142	0.02025	0.01633	0.01782	0.01368	0.00149	N											
BIS58	0.10742	0.11359	0.11406	0.11134	0.1134	0.11331	0.11423	0.02032	0.01713	0.01788	0.01218	0.00299	0.00298	N										
KAT49	0.10393	0.11155	0.11507	0.11136	0.11142	0.1121	0.11271	0.0225	0.01856	0.02079	0.01673	0.00449	0.00445	0.00598	N									
GIL72	0.11062	0.11472	0.11804	0.11507	0.11512	0.11581	0.11645	0.02698	0.02301	0.02376	0.02123	0.02086	0.02079	0.02084	0.02227	N								
GIL70	0.11062	0.11472	0.11804	0.11507	0.11512	0.11581	0.11645	0.02698	0.02301	0.02376	0.02123	0.02086	0.02079	0.02084	0.02227	0	N							
NIP50B	0.11062	0.11472	0.11804	0.11507	0.11512	0.11581	0.11645	0.02698	0.02301	0.02376	0.02123	0.02086	0.02079	0.02084	0.02227	0	0	N						
GIL44	0.10991	0.11401	0.11733	0.11436	0.1144	0.1151	0.11574	0.02625	0.02229	0.02303	0.02049	0.02013	0.02006	0.02011	0.02154	0	0	0	N					
ALO171	0.11328	0.11513	0.118242	0.11548	0.11534	0.11622	0.1169	0.0255	0.0216	0.02235	0.02129	0.02095	0.02087	0.02093	0.02238	0.00521	0.00521	0.00521	0.00447	N				
GIL45	0.10437	0.10214	0.11114	0.1225	0.12256	0.12325	0.13317	0.11929	0.11639	0.1194	0.12083	0.12154	0.1194	0.1214	0.11939	0.12389	0.12389	0.12389	0.12319	0.12017	N			
GIL89	0.10586	0.10364	0.11264	0.124	0.12406	0.12475	0.13317	0.12079	0.11788	0.12089	0.12083	0.12154	0.12089	0.1229	0.12089	0.12539	0.12539	0.12539	0.12469	0.12166	0.0015	N		
MER20	0.10468	0.10251	0.11136	0.12249	0.12255	0.12324	0.13306	0.11927	0.11581	0.11878	0.12094	0.1209	0.11878	0.12076	0.11878	0.12324	0.12324	0.12324	0.12254	0.11989	0	0.00149	N	
GIL57A	0.10468	0.10251	0.11136	0.12249	0.12255	0.12324	0.13306	0.11927	0.11581	0.11878	0.12094	0.1209	0.11878	0.12076	0.11878	0.12324	0.12324	0.12324	0.12254	0.11989	0	0.00149	0	N
RAM47B	0.10393	0.10326	0.11062	0.12324	0.1233	0.12398	0.13313	0.12003	0.11656	0.11952	0.12169	0.12164	0.11952	0.1215	0.11952	0.12398	0.12398	0.12398	0.12328	0.12064	0.00075	0.00224	0.00074	0.00074
SEM146	0.1069	0.10475	0.11284	0.12324	0.12329	0.12398	0.13382	0.11927	0.11581	0.11878	0.12094	0.1209	0.11878	0.12076	0.11878	0.12324	0.12324	0.12324	0.12254	0.11989	0.00376	0.00525	0.00371	0.00371
SEM152E	0.10393	0.10627	0.11359	0.12027	0.12033	0.12101	0.13231	0.12076	0.1173	0.12175	0.12248	0.1224	0.12027	0.12225	0.12027	0.12324	0.12324	0.12324	0.12254	0.12139	0.01652	0.01801	0.01633	0.01633
SEM160C	0.10472	0.10706	0.11437	0.12105	0.12111	0.12179	0.13238	0.12156	0.11809	0.12254	0.12251	0.12243	0.12106	0.12304	0.12106	0.12403	0.12403	0.12403	0.12333	0.12218	0.01727	0.01727	0.01708	0.01708
LAB53	0.10393	0.10625	0.11507	0.1173	0.11735	0.11804	0.13	0.11704	0.11359	0.1173	0.1187	0.11789	0.1173	0.11776	0.11581	0.12027	0.12027	0.12027	0.11956	0.11691	0.02857	0.03006	0.02895	0.02895
MesspG	0.10252	0.10332	0.10257	0.11439	0.11445	0.11514	0.12478	0.1239	0.12035	0.12109	0.12035	0.118	0.11886	0.11936	0.11738	0.1196	0.1196	0.1196	0.11889	0.12074	0.08872	0.09021	0.08839	0.08839
NIP66	0.10245	0.11249	0.10765	0.10542	0.10546	0.10616	0.12164	0.1193	0.11581	0.11581	0.11795	0.1179	0.1173	0.11778	0.11878	0.12101	0.12101	0.12101	0.12104	0.1184	0.11037	0.11186	0.10913	0.10913
GIL56	0.10296	0.11301	0.10813	0.10591	0.10596	0.10586	0.12173	0.11906	0.11636	0.11637	0.11799	0.11795	0.11786	0.11782	0.11936	0.12156	0.12156	0.12156	0.12159	0.11896	0.11086	0.11236	0.10961	0.10961
FUS2	0.10245	0.11249	0.10765	0.10542	0.10546	0.10616	0.12164	0.1193	0.11581	0.11581	0.11795	0.1179	0.1173	0.11778	0.11878	0.12101	0.12101	0.12101	0.12104	0.1184	0.11037	0.11186	0.10913	0.10913
FUS3	0.10245	0.11249	0.10765	0.10542	0.10546	0.10616	0.12164	0.1193	0.11581	0.11581	0.11795	0.1179	0.1173	0.11778	0.11878	0.12101	0.12101	0.12101	0.12104	0.1184	0.11037	0.11186	0.10913	0.10913
BAT39	0.10171	0.11325	0.10839	0.10468	0.10472	0.10542	0.1224	0.12005	0.11656	0.11656	0.11871	0.11865	0.11804	0.11852	0.11952	0.12175	0.12175	0.12175	0.12178	0.11914	0.10962	0.11111	0.10839	0.10839
GIL38	0.10187	0.11176	0.10629	0.10487	0.10492	0.10561	0.1211	0.11872	0.11524	0.11524	0.11739	0.11734	0.11673	0.11721	0.11821	0.12045	0.12045	0.12045	0.12048	0.11783	0.10899	0.11048	0.10776	0.10776
SEM159D	0.10468	0.11622	0.11136	0.10839	0.10844	0.10913	0.1247	0.11929	0.11581	0.11581	0.11796	0.1179	0.1173	0.11777	0.11878	0.12101	0.12101	0.12101	0.12104	0.1184	0.11112	0.11261	0.10987	0.10987
LAB81	0.1068	0.11216	0.11944	0.10451	0.10456	0.10448	0.11417	0.11842	0.11572	0.11796	0.12189	0.11875	0.1187	0.12019	0.11871	0.12167	0.12167	0.12167	0.12097	0.12058	0.10647	0.10647	0.10523	0.10523
LAY55	0.10616	0.11151	0.11878	0.10393	0.10398	0.10468	0.11408	0.11853	0.11507	0.1173	0.12184	0.11868	0.11804	0.12004	0.11804	0.12101	0.12101	0.12101	0.12031	0.11992	0.10591	0.1074	0.10468	0.10468
LAB83	0.10701	0.11239	0.11979	0.10471	0.10475	0.10467	0.1145	0.11892	0.11677	0.11906	0.12181	0.11985	0.11981	0.12132	0.11982	0.12277	0.12277	0.12277	0.12206	0.12133	0.10672	0.10672	0.10687	0.10687
LAB79	0.10778	0.11316	0.12055	0.10556	0.1056	0.10631	0.11438	0.12046	0.11753	0.11981	0.12175	0.11972	0.12056	0.12261	0.12057	0.12353	0.12353	0.12353	0.12282	0.12208	0.1076	0.1061	0.10774	0.10774
LAB74	0.10616	0.11151	0.11878	0.10393	0.10398	0.10468	0.11408	0.11853	0.11507	0.1173	0.12184	0.11868	0.11804	0.12004	0.11804	0.12101	0.12101	0.12101	0.12031	0.11992	0.10591	0.1074	0.10468	0.10468
LAB76	0.10616	0.11151	0.11878	0.10393	0.10398	0.10468	0.11408	0.11853	0.11507	0.1173	0.12184	0.11868	0.11804	0.12004	0.11804	0.12101	0.12101	0.12101	0.12031	0.11992	0.10591	0.1074	0.10468	0.10468
LAY86	0.11145	0.11181	0.12953	0.10538	0.10542	0.10536	0.11906	0.12263	0.12125	0.12052	0.12028	0.11825	0.11975	0.11973	0.12127	0.12195	0.12195	0.12195	0.12124	0.122	0.11496	0.11496	0.11513	0.11513
MER19	0.10987	0.11104	0.12769	0.10393	0.10397	0.10468	0.11789	0.12007	0.1173	0.11656	0.118	0.11643	0.1173	0.11779	0.11878	0.11952	0.11952	0.11952	0.11882	0.1199	0.11346	0.11496	0.1121	0.1121
CHE140F	0.10913	0.11104	0.12546	0.10468	0.10472	0.10542	0.11713	0.12006	0.1173	0.11656	0.11801	0.11494	0.11581	0.1163	0.1173	0.11952	0.11952	0.11952	0.11882	0.11991	0.11423	0.11572	0.11284	0.11284
CHE5	0.10913	0.11104	0.12546	0.10468	0.10472	0.10542	0.11713	0.12006	0.1173	0.11656	0.11801	0.11494	0.11581	0.1163	0.1173	0.11952	0.11952	0.11952	0.11882	0.11991	0.11423	0.11572	0.11284	0.11284
MesspD	0.10586	0.10695	0.1222	0.10434	0.10438	0.10431	0.11868	0.12201	0.11999	0.11925	0.11878	0.1157	0.11702	0.11706	0.11852	0.12222	0.12222	0.12222	0.12151	0.12262	0.1086	0.1101	0.10729	0.10729
POR34	0.10245	0.11158	0.11878	0.10913	0.10917	0.10987	0.12018	0.12381	0.12027	0.12175	0.11952	0.12016	0.11952	0.12003	0.11952	0.12101	0.12101	0.12101	0.1203	0.12139	0.11346	0.11495	0.1121	0.1121
CHE143A	0.10245	0.11158	0.11878	0.10913	0.10917	0.10987	0.12018	0.12381	0.12027	0.12175	0.11952	0.12016	0.11952	0.12003	0.11952	0.12101	0.12101	0.12101	0.1203	0.12139	0.11346	0.11495	0.1121	0.1121
CHE138C	0.10245	0.11158	0.11878	0.10913	0.10917	0.10987	0.12018	0.12381	0.12027	0.12175	0.11952	0.12016	0.11952	0.12003	0.11952	0.12101	0.12101	0.12101	0.1203	0.12139	0.11346	0.11495	0.1121	0.1121

Appendix C (continued)

	Ajapon	R.fungi	H.saxicola	USA37	USA36	RalfspB	RalfspC	NexpDes	CHE136	KEM114	LAY85	BES40	BIS73	BIS58	KAT49	GIL72	GIL70	NIP50B	GIL44	ALO171	GIL45	GIL89	MER20	GIL57A
TKS127A	0.10171	0.11084	0.11804	0.10839	0.10843	0.10913	0.11943	0.12306	0.11952	0.12101	0.11944	0.11941	0.11878	0.11929	0.11878	0.12027	0.12027	0.12027	0.11956	0.12065	0.11271	0.1142	0.11136	0.11136
MesspC	0.10097	0.10938	0.11952	0.10616	0.1062	0.10542	0.11565	0.11857	0.1173	0.11878	0.11879	0.11642	0.11656	0.11629	0.11656	0.11804	0.11804	0.11804	0.11733	0.11843	0.11569	0.11719	0.11433	0.11433
CHD117A	0.10171	0.11074	0.11284	0.10393	0.10399	0.10468	0.11721	0.11708	0.11433	0.11656	0.11735	0.11274	0.11359	0.1156	0.11359	0.11952	0.11952	0.11952	0.11882	0.11994	0.10742	0.10891	0.1069	0.1069
CHD122A	0.10171	0.11074	0.11284	0.10393	0.10399	0.10468	0.11721	0.11708	0.11433	0.11656	0.11735	0.11274	0.11359	0.1156	0.11359	0.11952	0.11952	0.11952	0.11882	0.11994	0.10742	0.10891	0.1069	0.1069
CHD120	0.10171	0.11074	0.11284	0.10393	0.10399	0.10468	0.11721	0.11708	0.11433	0.11656	0.11735	0.11274	0.11359	0.1156	0.11359	0.11952	0.11952	0.11952	0.11882	0.11994	0.10742	0.10891	0.1069	0.1069
KU1065	0.10171	0.11074	0.11433	0.10393	0.10399	0.10468	0.11872	0.11859	0.11581	0.11804	0.11887	0.11423	0.11507	0.11709	0.11507	0.12101	0.12101	0.12101	0.1203	0.12143	0.10742	0.10891	0.1069	0.1069
BIS62	0.09635	0.1017	0.10971	0.09704	0.09708	0.09702	0.10967	0.11622	0.11496	0.11721	0.1174	0.11277	0.11348	0.11422	0.11424	0.11868	0.11868	0.11868	0.11797	0.11985	0.10802	0.10802	0.10746	0.10746
BIS63	0.09651	0.10186	0.10987	0.09725	0.09729	0.098	0.11036	0.11712	0.11507	0.1173	0.11814	0.1135	0.11359	0.11486	0.11433	0.11878	0.11878	0.11878	0.11808	0.11994	0.10822	0.10971	0.10765	0.10765
SEM159A	0.09874	0.10707	0.11433	0.09874	0.09878	0.09948	0.11033	0.11486	0.11136	0.1121	0.11127	0.10826	0.10913	0.11113	0.10765	0.11359	0.11359	0.11359	0.11288	0.11473	0.10445	0.10594	0.10393	0.10393
SEM161B	0.09672	0.10501	0.11303	0.09744	0.09748	0.09818	0.10832	0.11423	0.11082	0.11156	0.11071	0.10771	0.10859	0.11059	0.10711	0.11304	0.11304	0.11304	0.11234	0.11419	0.10242	0.10391	0.10193	0.10193
MER14	0.09131	0.1048	0.1121	0.09354	0.09361	0.09428	0.10889	0.11258	0.10987	0.11062	0.11281	0.10829	0.10839	0.10966	0.10765	0.11062	0.11062	0.11062	0.10991	0.11178	0.10292	0.10441	0.10245	0.10245
MER15	0.09131	0.1048	0.1121	0.09354	0.09361	0.09428	0.10889	0.11183	0.10913	0.10987	0.11206	0.10755	0.10765	0.10892	0.1069	0.10987	0.10987	0.10987	0.10917	0.11104	0.10292	0.10441	0.10245	0.10245
CHE1	0.10177	0.1162	0.11438	0.09953	0.09959	0.10027	0.10967	0.11185	0.10913	0.10987	0.11203	0.10602	0.1069	0.1089	0.1069	0.10987	0.10987	0.10987	0.10917	0.11102	0.11051	0.112	0.10993	0.10993
CHD117B	0.098	0.10789	0.1121	0.10022	0.10028	0.10097	0.11105	0.11406	0.11136	0.10913	0.11501	0.11048	0.11136	0.11185	0.10839	0.1121	0.1121	0.1121	0.11139	0.11025	0.10444	0.10593	0.10319	0.10319
CHD122B	0.098	0.10789	0.1121	0.10022	0.10028	0.10097	0.11105	0.11406	0.11136	0.10913	0.11501	0.11048	0.11136	0.11185	0.10839	0.1121	0.1121	0.1121	0.11139	0.11025	0.10444	0.10593	0.10319	0.10319
KEM111	0.098	0.10789	0.1121	0.10022	0.10028	0.10097	0.11105	0.11406	0.11136	0.10913	0.11501	0.11048	0.11136	0.11185	0.10839	0.1121	0.1121	0.1121	0.11139	0.11025	0.10444	0.10593	0.10319	0.10319
KEM115	0.098	0.10789	0.1121	0.10022	0.10028	0.10097	0.11105	0.11406	0.11136	0.10913	0.11501	0.11048	0.11136	0.11185	0.10839	0.1121	0.1121	0.1121	0.11139	0.11025	0.10444	0.10593	0.10319	0.10319
KEM112	0.098	0.10789	0.1121	0.10022	0.10028	0.10097	0.11105	0.11406	0.11136	0.10913	0.11501	0.11048	0.11136	0.11185	0.10839	0.1121	0.1121	0.1121	0.11139	0.11025	0.10444	0.10593	0.10319	0.10319
KEM113	0.098	0.10789	0.1121	0.10022	0.10028	0.10097	0.11105	0.11406	0.11136	0.10913	0.11501	0.11048	0.11136	0.11185	0.10839	0.1121	0.1121	0.1121	0.11139	0.11025	0.10444	0.10593	0.10319	0.10319
LEN41	0.09782	0.10917	0.11269	0.10226	0.10231	0.10223	0.11268	0.11543	0.11347	0.11124	0.11656	0.11202	0.11347	0.11347	0.11051	0.1142	0.1142	0.1142	0.11349	0.11235	0.10498	0.10498	0.10373	0.10373
GIL57B	0.098	0.10791	0.1121	0.09948	0.09953	0.10022	0.10953	0.11406	0.11136	0.10913	0.11492	0.10972	0.11136	0.11185	0.10839	0.1121	0.1121	0.1121	0.11139	0.11025	0.10593	0.10593	0.10468	0.10468
CHE140D	0.10326	0.11322	0.11214	0.10548	0.10534	0.10622	0.11492	0.1171	0.11437	0.11363	0.11651	0.11274	0.11438	0.11487	0.11141	0.11511	0.11511	0.11511	0.1144	0.11309	0.10895	0.10895	0.10768	0.10768
GIL88	0.09725	0.10316	0.11359	0.10022	0.10028	0.10097	0.11259	0.11482	0.1121	0.11062	0.11272	0.10898	0.10913	0.10961	0.10616	0.11136	0.11136	0.11136	0.11065	0.11175	0.10218	0.10367	0.10097	0.10097
MER10	0.11433	0.1147	0.12621	0.1121	0.11214	0.11284	0.12765	0.12308	0.12101	0.12027	0.11942	0.11938	0.11878	0.11924	0.12027	0.12398	0.12398	0.12398	0.12327	0.1236	0.11269	0.11418	0.1121	0.1121
MER17	0.11359	0.11548	0.12398	0.11359	0.11363	0.11433	0.12917	0.12309	0.12101	0.12027	0.11941	0.11789	0.1173	0.11775	0.11878	0.12398	0.12398	0.12398	0.12327	0.1236	0.11344	0.11493	0.11284	0.11284
SEM162	0.11267	0.1116	0.12527	0.11412	0.11417	0.11408	0.1262	0.1229	0.12159	0.12084	0.12101	0.12094	0.12085	0.12081	0.12235	0.12604	0.12604	0.12604	0.12534	0.12494	0.1102	0.1117	0.10962	0.10962
SEM165	0.1121	0.11109	0.12472	0.11359	0.11363	0.11433	0.1261	0.12311	0.12101	0.12027	0.12096	0.12088	0.12027	0.12075	0.12175	0.12546	0.12546	0.12546	0.12476	0.12435	0.1097	0.1112	0.10913	0.10913
SEM163	0.1121	0.11109	0.12472	0.11359	0.11363	0.11433	0.1261	0.12311	0.12101	0.12027	0.12096	0.12088	0.12027	0.12075	0.12175	0.12546	0.12546	0.12546	0.12476	0.12435	0.1097	0.1112	0.10913	0.10913
SEM164	0.1121	0.11109	0.12472	0.11359	0.11363	0.11433	0.1261	0.12311	0.12101	0.12027	0.12096	0.12088	0.12027	0.12075	0.12175	0.12546	0.12546	0.12546	0.12476	0.12435	0.1097	0.1112	0.10913	0.10913
SEM166	0.1121	0.11109	0.12472	0.11359	0.11363	0.11433	0.1261	0.12311	0.12101	0.12027	0.12096	0.12088	0.12027	0.12075	0.12175	0.12546	0.12546	0.12546	0.12476	0.12435	0.1097	0.1112	0.10913	0.10913
DAN78	0.10771	0.11341	0.12687	0.11005	0.11009	0.11081	0.12443	0.12447	0.12151	0.12074	0.12147	0.12292	0.12228	0.12278	0.12229	0.12304	0.12304	0.12304	0.12231	0.12229	0.1099	0.11143	0.11005	0.11005
DAN77	0.10857	0.11407	0.12639	0.11079	0.11083	0.11154	0.12478	0.12387	0.1212	0.12046	0.12096	0.12255	0.12194	0.12242	0.12195	0.12268	0.12268	0.12268	0.12197	0.12213	0.10972	0.11121	0.10929	0.10929
TKA109	0.10839	0.11389	0.12621	0.11062	0.11066	0.11136	0.12457	0.12385	0.12101	0.12027	0.12094	0.12236	0.12175	0.12223	0.12175	0.12249	0.12249	0.12249	0.12179	0.12211	0.10971	0.1112	0.10913	0.10913
TKA110	0.10839	0.11389	0.12621	0.11062	0.11066	0.11136	0.12457	0.12385	0.12101	0.12027	0.12094	0.12236	0.12175	0.12223	0.12175	0.12249	0.12249	0.12249	0.12179	0.12211	0.10971	0.1112	0.10913	0.10913
TKS130	0.10839	0.11389	0.12621	0.11062	0.11066	0.11136	0.12457	0.12385	0.12101	0.12027	0.12094	0.12236	0.12175	0.12223	0.12175	0.12249	0.12249	0.12249	0.12179	0.12211	0.10971	0.1112	0.10913	0.10913
TKS129	0.10765	0.11388	0.12621	0.11062	0.11066	0.11136	0.12459	0.12535	0.12249	0.12175	0.12247	0.12386	0.12324	0.12372	0.12175	0.12398	0.12398	0.12398	0.12327	0.1236	0.11121	0.1127	0.11062	0.11062
TKS131	0.10765	0.11314	0.12546	0.10987	0.10992	0.11062	0.12382	0.1246	0.12175	0.12101	0.1217	0.12311	0.12249	0.12297	0.12101	0.12324	0.12324	0.12324	0.12253	0.12286	0.11046	0.11195	0.10987	0.10987
BAT43	0.10765	0.11314	0.12546	0.10987	0.10992	0.11062	0.12382	0.1246	0.12175	0.12101	0.1217	0.12311	0.12249	0.12297	0.12101	0.12324	0.12324	0.12324	0.12253	0.12286	0.11046	0.11195	0.10987	0.10987
POR96	0.10641	0.10889	0.12357	0.10869	0.10874	0.10944	0.12344	0.12184	0.11904	0.11829	0.11895	0.11964	0.11903	0.11951	0.11903	0.12053	0.12053	0.12053	0.11982	0.12015	0.10769	0.10918	0.10715	0.10715
TKR133	0.11062	0.111	0.12843	0.11136	0.1114	0.1121	0.12538	0.12234	0.11952	0.11878	0.11945	0.11939	0.11878	0.11926	0.11878	0.12249	0.12249	0.12249	0.12179	0.12138	0.10895	0.11045	0.10839	0.10839
TKS127B	0.11062	0.111	0.12843	0.11136	0.1114	0.1121	0.12538	0.12234	0.11952	0.11878	0.11945	0.11939	0.11878	0.11926	0.11878	0.12249	0.12249	0.12249	0.12179	0.12138	0.10895	0.11045	0.10839	0.10839
BAT7A	0.11062	0.11013	0.12843	0.11136	0.1114	0.1121	0.12766	0.12383	0.12101	0.12027	0.12097	0.12089	0.12027	0.12075	0.12027	0.12398	0.12398	0.12398	0.12327	0.12287	0.10894	0.11043	0.10839	0.10839
POR32	0.11139	0.10936	0.12626	0.11209	0.11214	0.11206	0.12726	0.12372	0.12182	0.12108	0.12106	0.12116	0.12109	0.12105	0.1211	0.1248	0.1248	0.1248	0.12409	0.12351	0.10872	0.10949	0.10834	0.10834
MER18	0.11062	0.10863	0.12546	0.11136	0.11141	0.1121	0.12693	0.12383	0.12101	0.12027	0.12098	0.12089	0.12027	0.12075	0.12027	0.12398	0.12398	0.12398	0.12327	0.12287	0.10818	0.10967	0.10765	0.10765
MER11	0.11213	0.11012	0.127	0.11284	0.11289	0.11281	0.12799	0.12454	0.12257	0.12182	0.12182	0.12184	0.12183	0.1218	0.12184	0.12554	0.12554	0.12554	0.12483	0.12432	0.10801	0.10801	0.10759	0.10759
MER71	0.11062	0.10863	0.12546	0.11136	0.11141	0.1121	0.12693	0.12383	0.12101	0.12027	0.12098	0.12089	0.12027	0.12075	0.12027	0.12398	0.12398	0.12398	0.12327	0.12287	0.10818	0.10967	0.10765	0.10765
PTL35	0.11062	0.10863	0.12546	0.11136	0.11141	0.1121	0.12693	0.12383	0.12101	0.12027	0.12098	0.12089	0.12027	0.12075	0.12027	0.12398	0.12398	0.12398	0.12327	0.12287	0.10818	0.10967	0.10765	0.10765
MER16	0.11085	0.10877	0.12583	0.11089	0.11094	0.11086	0.12618	0.1235	0.12136	0.12063	0.12085	0.12073	0.12062	0.1206	0.12063	0.12435	0.12435	0.12435	0.12365	0.12289	0.10853	0.11004	0.10855	0.10855
MER13	0.10943	0.1074	0.12507	0.11016	0.11021	0.1109	0.12577	0.12247	0.11983	0.11908	0.1196	0.11971	0.11909	0.11958	0.11909	0.1228	0.1228	0.1228	0.12209	0.12151	0.10758	0.10908	0.10721	0.10721
POR31	0.1069	0.10637	0.12472	0.10839	0.10844	0.10913	0.12391	0.12007	0.1173	0.11656	0.11718	0.11717	0.11656	0.11703	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10742	0.10542	0.10542
CHE140C	0.10754	0.10698	0.1254	0.10901	0.10905	0.10898	0.12407	0.12001	0.11798	0.11724	0.11726	0.11725	0.11724	0.11721	0.11726	0.12096	0.12096	0.12096	0.12025	0.11984	0.10651	0.10651	0.106	0.106

Appendix C (continued)

	A.japon	R.fungi	H.saxicola	USA37	USA36	RalfspB	RalfspC	NexpDes	CHE136	KEM114	LAY85	BES40	BIS73	BIS58	KAT49	GIL72	GIL70	NIP50B	GIL44	ALO171	GIL45	GIL89	MER20	GIL57A
POR168	0.1069	0.10637	0.12472	0.10839	0.10844	0.10913	0.12391	0.12007	0.1173	0.11656	0.11718	0.11717	0.11656	0.11703	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10742	0.10542	0.10542
CHE139A	0.1069	0.10637	0.12472	0.10839	0.10844	0.10913	0.12391	0.12007	0.1173	0.11656	0.11718	0.11717	0.11656	0.11703	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10742	0.10542	0.10542
SEM147	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
SEM161A	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
KUN148	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
KUN149	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
SEM151	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
SEM152A	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
SEM156	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
SEM144C	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
SEM158A	0.1069	0.10564	0.12472	0.10913	0.10918	0.10987	0.12468	0.12006	0.1173	0.11656	0.1172	0.11717	0.11656	0.11704	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10741	0.10542	0.10542
SEM160A	0.10616	0.1064	0.12398	0.10839	0.10844	0.10913	0.12391	0.12082	0.11804	0.1173	0.11795	0.11792	0.1173	0.11778	0.1173	0.11952	0.11952	0.11952	0.11882	0.11915	0.10667	0.10817	0.10616	0.10616
MER23	0.10629	0.10568	0.12434	0.10785	0.10789	0.1086	0.12362	0.12049	0.11757	0.11684	0.11708	0.11746	0.11683	0.11733	0.11683	0.12056	0.12056	0.12056	0.11985	0.1191	0.10614	0.10765	0.10626	0.10626
CHE3	0.10628	0.1057	0.12445	0.10781	0.10786	0.10779	0.12315	0.11985	0.11767	0.11692	0.11725	0.11701	0.11692	0.1169	0.11694	0.12068	0.12068	0.12068	0.11996	0.1192	0.10612	0.1069	0.10625	0.10625
MER22	0.10782	0.10728	0.12564	0.1093	0.10935	0.11004	0.12409	0.12083	0.11822	0.11748	0.11796	0.1181	0.11748	0.11796	0.11748	0.12119	0.12119	0.12119	0.12049	0.1199	0.10667	0.10816	0.10632	0.10632
CHE142	0.1069	0.10637	0.12472	0.10839	0.10844	0.10913	0.12391	0.12007	0.1173	0.11656	0.11718	0.11717	0.11656	0.11703	0.11656	0.12027	0.12027	0.12027	0.11956	0.11915	0.10592	0.10742	0.10542	0.10542
CHE138E	0.10254	0.10828	0.12117	0.09931	0.09936	0.1001	0.11222	0.1188	0.11578	0.11655	0.1158	0.11726	0.11657	0.11713	0.1166	0.12194	0.12194	0.12194	0.12121	0.12045	0.1086	0.11016	0.10875	0.10875
Hap.saxi	0.10319	0.10569	0.12101	0.11062	0.11066	0.11136	0.11867	0.12079	0.11656	0.11656	0.11946	0.1194	0.11878	0.11926	0.11878	0.11656	0.11656	0.11656	0.11584	0.11469	0.12172	0.12321	0.12101	0.12101
LAY54	0.12019	0.1165	0.12776	0.12103	0.12104	0.12178	0.13285	0.13292	0.13005	0.13156	0.12899	0.12838	0.12779	0.12673	0.12628	0.13153	0.13153	0.13153	0.13082	0.13149	0.12835	0.12986	0.1286	0.1286
Nem.ting	0.13986	0.14951	0.15424	0.14823	0.14826	0.14819	0.15241	0.16591	0.16352	0.16438	0.16437	0.16354	0.16437	0.16265	0.16271	0.16178	0.16178	0.16178	0.161	0.1626	0.17265	0.17265	0.17284	0.17284
Heri.fluv	0.13188	0.14124	0.15519	0.14619	0.14624	0.14695	0.14621	0.1581	0.15446	0.15597	0.15905	0.15619	0.15522	0.15679	0.15598	0.16272	0.16272	0.16272	0.16202	0.16205	0.16481	0.16632	0.16496	0.16496
Pse.roscf	0.15987	0.1557	0.17064	0.15567	0.15569	0.15562	0.1474	0.16221	0.15907	0.16323	0.15995	0.1608	0.15996	0.15913	0.16081	0.16237	0.16237	0.16237	0.16161	0.1632	0.17457	0.17457	0.17477	0.17477
MER91	0.16036	0.16519	0.17298	0.1663	0.16635	0.1663	0.17068	0.17025	0.16927	0.17223	0.17389	0.17109	0.17001	0.17013	0.16852	0.17149	0.17149	0.17149	0.17081	0.17364	0.18095	0.18244	0.1804	0.1804
MER95	0.16036	0.16519	0.17298	0.1663	0.16635	0.1663	0.17068	0.17025	0.16927	0.17223	0.17389	0.17109	0.17001	0.17013	0.16852	0.17149	0.17149	0.17149	0.17081	0.17364	0.18095	0.18244	0.1804	0.1804
CHE6	0.16036	0.16519	0.17298	0.1663	0.16635	0.1663	0.17068	0.17025	0.16927	0.17223	0.17389	0.17109	0.17001	0.17013	0.16852	0.17149	0.17149	0.17149	0.17081	0.17364	0.18095	0.18244	0.1804	0.1804
CHE7	0.16036	0.16519	0.17298	0.1663	0.16635	0.1663	0.17068	0.17025	0.16927	0.17223	0.17389	0.17109	0.17001	0.17013	0.16852	0.17149	0.17149	0.17149	0.17081	0.17364	0.18095	0.18244	0.1804	0.1804
POR100	0.16036	0.16519	0.17298	0.1663	0.16635	0.1663	0.17068	0.17025	0.16927	0.17223	0.17389	0.17109	0.17001	0.17013	0.16852	0.17149	0.17149	0.17149	0.17081	0.17364	0.18095	0.18244	0.1804	0.1804
MER21	0.15961	0.16295	0.17223	0.16407	0.16413	0.16481	0.17078	0.16873	0.16704	0.17001	0.1724	0.16964	0.16778	0.16867	0.1663	0.16927	0.16927	0.16927	0.16858	0.17141	0.17868	0.18017	0.17817	0.17817
POR27	0.16036	0.1637	0.17298	0.16481	0.16487	0.16555	0.17084	0.16948	0.16778	0.17075	0.17316	0.17038	0.16852	0.16942	0.16704	0.17001	0.17001	0.17001	0.16932	0.17216	0.17943	0.18093	0.17892	0.17892
CHE4	0.15966	0.16301	0.17228	0.16411	0.16417	0.16485	0.17083	0.16872	0.16704	0.17001	0.17238	0.16963	0.16778	0.16867	0.1663	0.16927	0.16927	0.16927	0.16858	0.17141	0.17872	0.18021	0.17822	0.17822
Por.fluv	0.13511	0.1368	0.16003	0.14021	0.14029	0.14125	0.14424	0.16224	0.16037	0.16216	0.16083	0.15854	0.15716	0.15719	0.15628	0.1604	0.1604	0.1604	0.15942	0.16154	0.18175	0.18381	0.18187	0.18187
Petro.mac	0.14334	0.15216	0.16421	0.1549	0.15493	0.15413	0.15439	0.16719	0.16501	0.16503	0.16617	0.16997	0.16891	0.17055	0.16971	0.16811	0.16811	0.16811	0.1674	0.16735	0.17882	0.18038	0.17898	0.17898
Dipsimplx	0.17019	0.16929	0.18387	0.18616	0.18622	0.18529	0.19168	0.18924	0.1886	0.19023	0.18465	0.18632	0.18506	0.18333	0.1815	0.18695	0.18695	0.18695	0.18612	0.18699	0.1882	0.18993	0.18835	0.18835
DipspB	0.18634	0.18919	0.20267	0.20267	0.20273	0.20341	0.20879	0.2045	0.2049	0.20638	0.19719	0.20244	0.20045	0.19993	0.19822	0.20341	0.20341	0.20341	0.20274	0.201	0.19716	0.19866	0.20119	0.20119
DipspC	0.16184	0.16234	0.17743	0.18263	0.18268	0.18337	0.189	0.18302	0.17892	0.1804	0.17764	0.17704	0.1752	0.17457	0.17298	0.17743	0.17743	0.17743	0.17673	0.17584	0.17789	0.17938	0.17817	0.17817
POR169	0.17619	0.173	0.18707	0.17929	0.17933	0.17922	0.18313	0.18585	0.18231	0.18233	0.1799	0.18085	0.17999	0.17999	0.17847	0.18232	0.18232	0.18232	0.18161	0.18148	0.19227	0.19227	0.19307	0.19307
TKS126	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
POR26	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
CHD121	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
CHD123	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
POR29	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
POR24	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
CHD118	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
TKS128	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
POR33	0.1752	0.17057	0.18634	0.17743	0.17748	0.17817	0.18463	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
POR94	0.17446	0.16981	0.1856	0.17817	0.17822	0.17892	0.18387	0.18311	0.17892	0.17892	0.17774	0.17856	0.17669	0.1776	0.1752	0.17966	0.17966	0.17966	0.17897	0.17884	0.1885	0.19	0.18931	0.18931
MER8	0.17446	0.16981	0.1856	0.17817	0.17822	0.17892	0.18387	0.18311	0.17892	0.17892	0.17774	0.17856	0.17669	0.1776	0.1752	0.17966	0.17966	0.17966	0.17897	0.17884	0.1885	0.19	0.18931	0.18931
GIL42	0.1752	0.17055	0.18634	0.17892	0.17896	0.17966	0.18397	0.18386	0.17966	0.17966	0.1785	0.17931	0.17743	0.17835	0.17595	0.1804	0.1804	0.1804	0.17971	0.17959	0.18925	0.19075	0.19005	0.19005
SEM155	0.17446	0.16981	0.1856	0.17743	0.17748	0.17817	0.18311	0.1831	0.17892	0.17892	0.17774	0.17857	0.17669	0.17761	0.1752	0.17966	0.17966	0.17966	0.17897	0.17885	0.18925	0.19075	0.19005	0.19005
LAB46	0.17002	0.16782	0.177443	0.17669	0.17674	0.17744	0.18122	0.18228	0.17891	0.17892	0.17747	0.17693	0.17593	0.17754	0.1752	0.17741	0.17741	0.17741	0.17671	0.17818	0.18244	0.18244	0.18335	0.18335
RAM65	0.17002	0.16981	0.1856	0.17743	0.17748	0.17817	0.18122	0.18228	0.17891	0.17892	0.17747	0.17693	0.17593	0.17754	0.1752	0.17741	0.17741	0.17741	0.17671	0.17818	0.18244	0.18244	0.18335	0.18335

Appendix C (continued)

	A.japon	R.fungi	H.saxicola	USA37	USA36	RalfspB	RalfspC	NexpDes	CHE136	KEM114	LAY85	BES40	BIS73	BIS58	KAT49	GIL72	GIL70	NIP50B	GIL44	ALO171	GIL45	GIL89	MER20	GIL57A
LAB80	0.16853	0.16782	0.177443	0.17669	0.17674	0.17744	0.18126	0.18078	0.17742	0.17742	0.17678	0.17625	0.17444	0.17604	0.1737	0.17591	0.17591	0.17591	0.17521	0.17669	0.18093	0.18243	0.18186	0.18186
RAM47A	0.16808	0.16588	0.17403	0.1748	0.17484	0.17555	0.18085	0.18071	0.17704	0.17703	0.17672	0.17584	0.17404	0.17564	0.17329	0.17553	0.17553	0.17553	0.17483	0.17631	0.18087	0.18237	0.18152	0.18152
LAB82	0.16793	0.16572	0.17385	0.17463	0.17467	0.17537	0.18067	0.18076	0.17685	0.17686	0.17676	0.17567	0.17387	0.17547	0.17312	0.17535	0.17535	0.17535	0.17465	0.17613	0.18091	0.18241	0.18131	0.18131
LAB84	0.16739	0.16521	0.17336	0.17491	0.17495	0.17566	0.18097	0.18071	0.17714	0.17714	0.17671	0.17595	0.17415	0.17575	0.17339	0.17563	0.17563	0.17563	0.17493	0.17642	0.18086	0.18236	0.18094	0.18094
LAB75	0.16703	0.16481	0.17293	0.17445	0.1745	0.1752	0.1814	0.17928	0.17592	0.17593	0.17526	0.17474	0.17294	0.17455	0.17221	0.17442	0.17442	0.17442	0.17372	0.1752	0.17944	0.18093	0.18036	0.18036
CHE143C	0.1766	0.18633	0.19334	0.1929	0.19291	0.19285	0.19874	0.19406	0.18981	0.18855	0.19346	0.18637	0.18642	0.18723	0.18734	0.18977	0.18977	0.18977	0.18892	0.18767	0.19038	0.19038	0.19046	0.19046
CHE141	0.17149	0.17733	0.18411	0.19005	0.19007	0.19079	0.19737	0.19208	0.18782	0.19079	0.19211	0.18522	0.18337	0.18498	0.18263	0.18931	0.18931	0.18931	0.18861	0.18844	0.18244	0.18393	0.18263	0.18263
CHE139B	0.17149	0.17733	0.18411	0.19005	0.19007	0.19079	0.19737	0.19208	0.18782	0.19079	0.19211	0.18522	0.18337	0.18498	0.18263	0.18931	0.18931	0.18931	0.18861	0.18844	0.18244	0.18393	0.18263	0.18263
SAY134	0.17149	0.17733	0.18411	0.19005	0.19007	0.19079	0.19737	0.19208	0.18782	0.19079	0.19211	0.18522	0.18337	0.18498	0.18263	0.18931	0.18931	0.18931	0.18861	0.18844	0.18244	0.18393	0.18263	0.18263
SAY135	0.17149	0.17733	0.18411	0.19005	0.19007	0.19079	0.19737	0.19208	0.18782	0.19079	0.19211	0.18522	0.18337	0.18498	0.18263	0.18931	0.18931	0.18931	0.18861	0.18844	0.18244	0.18393	0.18263	0.18263
CHE137	0.17149	0.17733	0.18411	0.19005	0.19007	0.19079	0.19737	0.19208	0.18782	0.19079	0.19211	0.18522	0.18337	0.18498	0.18263	0.18931	0.18931	0.18931	0.18861	0.18844	0.18244	0.18393	0.18263	0.18263
SEM144E	0.14848	0.15937	0.16258	0.16555	0.16561	0.1663	0.17302	0.18306	0.17817	0.18263	0.18451	0.17853	0.17669	0.17606	0.17372	0.18189	0.18189	0.18189	0.18119	0.18106	0.17495	0.17644	0.17372	0.17372
POR99	0.14848	0.15937	0.16258	0.16555	0.16561	0.1663	0.17302	0.18306	0.17817	0.18263	0.18451	0.17853	0.17669	0.17606	0.17372	0.18189	0.18189	0.18189	0.18119	0.18106	0.17495	0.17644	0.17372	0.17372
POR167	0.14848	0.15937	0.16258	0.16555	0.16561	0.1663	0.17302	0.18306	0.17817	0.18263	0.18451	0.17853	0.17669	0.17606	0.17372	0.18189	0.18189	0.18189	0.18119	0.18106	0.17495	0.17644	0.17372	0.17372
MER64	0.14625	0.15713	0.16036	0.16407	0.16412	0.16481	0.17073	0.18082	0.17595	0.1804	0.18222	0.17629	0.17446	0.17383	0.17149	0.17966	0.17966	0.17966	0.17896	0.17883	0.17271	0.1742	0.17149	0.17149
LAY52	0.14848	0.15788	0.16258	0.16258	0.16263	0.16333	0.1715	0.18007	0.1752	0.17966	0.18147	0.17704	0.1752	0.17457	0.17223	0.17892	0.17892	0.17892	0.17822	0.17809	0.17346	0.17495	0.17223	0.17223
GIL67	0.14985	0.15934	0.16413	0.16271	0.16275	0.16346	0.17177	0.17978	0.1762	0.18074	0.18121	0.17808	0.1762	0.17559	0.17321	0.17996	0.17996	0.17996	0.17926	0.1785	0.17373	0.17524	0.17398	0.17398
LAY51	0.14848	0.15788	0.16258	0.16258	0.16263	0.16333	0.1715	0.18007	0.1752	0.17966	0.18147	0.17704	0.1752	0.17457	0.17223	0.17892	0.17892	0.17892	0.17822	0.17809	0.17346	0.17495	0.17223	0.17223
GIL48B	0.14848	0.15788	0.16258	0.16258	0.16263	0.16258	0.1715	0.18007	0.1752	0.17966	0.18147	0.17704	0.1752	0.17457	0.17223	0.17892	0.17892	0.17892	0.17822	0.17809	0.17346	0.17495	0.17223	0.17223
CHE2	0.14477	0.15563	0.16036	0.16258	0.16264	0.16258	0.1707	0.17932	0.17446	0.17892	0.1807	0.1748	0.17298	0.17233	0.17001	0.17817	0.17817	0.17817	0.17748	0.17734	0.1712	0.17269	0.17001	0.17001

Appendix C (continued)

	RAM47B	SEM146	SEM152E	SEM160C	LAB53	MesspG	NIP66	GIL56	FUS2	FUS3	BAT39	GIL38	SEM159D	LAB81	LAY55	LAB83	LAB79	LAB74	LAB76	LAY86	MER19	CHE140F	CHE5	MesspD
RAM47B	N																							
SEM146	0.00445	N																						
SEM152E	0.01707	0.01559	N																					
SEM160C	0.01783	0.01634	0.00074	N																				
LAB53	0.0297	0.03118	0.03638	0.03714	N																			
MesspG	0.08914	0.08988	0.0884	0.08918	0.08691	N																		
NIP66	0.10987	0.10987	0.10765	0.10843	0.10839	0.11371	N																	
GIL56	0.11036	0.11036	0.10812	0.1089	0.10886	0.11422	0	N																
FUS2	0.10987	0.10987	0.10765	0.10843	0.10839	0.11371	0	0	N															
FUS3	0.10987	0.10987	0.10765	0.10843	0.10839	0.11371	0	0	0	N														
BAT39	0.10913	0.10913	0.1069	0.10769	0.10765	0.11297	0.00074	0.00074	0.00074	0.00074	N													
GIL38	0.10851	0.1085	0.10629	0.10707	0.10703	0.11235	0	0	0	0	0.00075	N												
SEM159D	0.11062	0.11062	0.1069	0.10769	0.10913	0.11445	0.00742	0.00746	0.00742	0.00742	0.00817	0.00743	N											
LAB81	0.10597	0.10597	0.09926	0.09929	0.10297	0.10609	0.0716	0.07167	0.0716	0.0716	0.07234	0.0702	0.0701	N										
LAY55	0.10542	0.10542	0.09874	0.09952	0.10245	0.1055	0.07127	0.07158	0.07127	0.07127	0.07201	0.06987	0.06978	0	N									
LAB83	0.10762	0.10762	0.10082	0.10086	0.10385	0.10703	0.0719	0.07197	0.0719	0.0719	0.07265	0.07049	0.07038	0	0	N								
LAB79	0.10849	0.10849	0.10173	0.10101	0.10476	0.10785	0.07303	0.07335	0.07303	0.07303	0.07378	0.07162	0.07152	0	0.0015	0	N							
LAB74	0.10542	0.10542	0.09874	0.09952	0.10245	0.1055	0.07127	0.07158	0.07127	0.07127	0.07201	0.06987	0.06978	0	0	0	0.0015	N						
LAB76	0.10542	0.10542	0.09874	0.09952	0.10245	0.1055	0.07127	0.07158	0.07127	0.07127	0.07201	0.06987	0.06978	0	0	0	0.0015	0	N					
LAY86	0.11588	0.11589	0.11288	0.11213	0.11743	0.11829	0.09158	0.09163	0.09158	0.09158	0.09082	0.09102	0.0893	0.0773	0.07726	0.07735	0.07728	0.07726	0.07726	N				
MER19	0.11284	0.11284	0.10987	0.10991	0.11507	0.11811	0.08983	0.0902	0.08983	0.08983	0.08909	0.08927	0.0876	0.07533	0.07498	0.07648	0.07759	0.07498	0.07498	0.00227	N			
CHE140F	0.11359	0.11359	0.11062	0.11065	0.11581	0.11662	0.09057	0.09095	0.09057	0.09057	0.08983	0.09001	0.08834	0.07608	0.07572	0.07725	0.07835	0.07572	0.07572	0.00303	0.0052	N		
CHE5	0.11359	0.11359	0.11062	0.11065	0.11581	0.11662	0.09057	0.09095	0.09057	0.09057	0.08983	0.09001	0.08834	0.07608	0.07572	0.07725	0.07835	0.07572	0.07572	0.00303	0.0052	0	N	
MesspD	0.10804	0.10878	0.10581	0.10584	0.11251	0.11408	0.09312	0.09319	0.09312	0.09312	0.09237	0.09256	0.09089	0.07983	0.07973	0.08107	0.08245	0.07973	0.07973	0.01139	0.01192	0.01118	0.01118	N
POR34	0.11284	0.11284	0.10839	0.10917	0.11136	0.11588	0.07424	0.07456	0.07424	0.07424	0.07498	0.0744	0.07275	0.0731	0.07275	0.0742	0.07531	0.07275	0.07275	0.07266	0.07127	0.07127	0.07127	0.0745
CHE143A	0.11284	0.11284	0.10839	0.10917	0.11136	0.11588	0.07424	0.07456	0.07424	0.07424	0.07498	0.0744	0.07275	0.0731	0.07275	0.0742	0.07531	0.07275	0.07275	0.07266	0.07127	0.07127	0.07127	0.0745
CHE138C	0.11284	0.11284	0.10839	0.10917	0.11136	0.11588	0.07424	0.07456	0.07424	0.07424	0.07498	0.0744	0.07275	0.0731	0.07275	0.0742	0.07531	0.07275	0.07275	0.07266	0.07127	0.07127	0.07127	0.0745
TKS127A	0.1121	0.1121	0.10765	0.10842	0.11062	0.11514	0.07498	0.0753	0.07498	0.07498	0.07572	0.07514	0.0735	0.07385	0.0735	0.07496	0.07606	0.0735	0.0735	0.07342	0.07201	0.07201	0.07201	0.07525
MesspC	0.11507	0.11507	0.10913	0.10991	0.1121	0.11366	0.07906	0.07869	0.07869	0.07795	0.07732	0.07812	0.07498	0.07309	0.0735	0.07422	0.0761	0.0735	0.0735	0.07193	0.07127	0.06978	0.06978	0.07076
CHD117A	0.10765	0.10765	0.10245	0.10248	0.10987	0.10178	0.07869	0.07906	0.07869	0.07869	0.07795	0.07732	0.07424	0.0694	0.06904	0.06975	0.07085	0.06904	0.06904	0.06666	0.06533	0.06459	0.06459	0.07006
CHD122A	0.10765	0.10765	0.10245	0.10248	0.10987	0.10178	0.07869	0.07906	0.07869	0.07869	0.07795	0.07732	0.07424	0.0694	0.06904	0.06975	0.07085	0.06904	0.06904	0.06666	0.06533	0.06459	0.06459	0.07006
CHD120	0.10765	0.10765	0.10245	0.10248	0.10987	0.10178	0.07869	0.07906	0.07869	0.07869	0.07795	0.07732	0.07424	0.0694	0.06904	0.06975	0.07085	0.06904	0.06904	0.06666	0.06533	0.06459	0.06459	0.07006
KU1065	0.10765	0.10765	0.10245	0.10248	0.10839	0.10327	0.07869	0.07906	0.07869	0.07869	0.07795	0.07732	0.07275	0.0694	0.06904	0.06975	0.07085	0.06904	0.06904	0.06666	0.06533	0.06459	0.06459	0.07006
BIS62	0.1082	0.1082	0.10149	0.10076	0.11192	0.10459	0.07383	0.0739	0.07383	0.07383	0.07308	0.07245	0.07383	0.0717	0.07162	0.07212	0.07201	0.07162	0.07162	0.06901	0.06784	0.0671	0.0671	0.07088
BIS63	0.10839	0.10839	0.10171	0.10173	0.1121	0.10327	0.07275	0.07311	0.07275	0.07275	0.07201	0.07137	0.07275	0.0724	0.07201	0.07284	0.07391	0.07201	0.07201	0.06975	0.0683	0.06756	0.06756	0.07155
SEM159A	0.10468	0.10468	0.10097	0.10099	0.10468	0.1055	0.07647	0.07683	0.07647	0.07647	0.07572	0.07508	0.07647	0.07312	0.07275	0.07358	0.07467	0.07275	0.07275	0.06823	0.0683	0.06607	0.06607	0.07005
SEM161B	0.10267	0.10267	0.09896	0.09898	0.1027	0.10349	0.07371	0.07407	0.07371	0.07371	0.07297	0.07231	0.07371	0.07038	0.07002	0.0708	0.0719	0.07002	0.07002	0.06615	0.06626	0.06402	0.06402	0.06801
MER14	0.10319	0.10319	0.098	0.09802	0.10393	0.10104	0.07647	0.07685	0.07647	0.07647	0.07572	0.0751	0.07498	0.06944	0.06904	0.06981	0.07086	0.06904	0.06904	0.07051	0.06904	0.0683	0.0683	0.07232
MER15	0.10319	0.10319	0.098	0.09802	0.10393	0.10104	0.07647	0.07685	0.07647	0.07647	0.07572	0.0751	0.07498	0.06944	0.06904	0.06981	0.07086	0.06904	0.06904	0.07051	0.06904	0.0683	0.0683	0.07232
CHE1	0.11067	0.11067	0.10547	0.10549	0.11216	0.10555	0.07725	0.07762	0.07725	0.07725	0.07799	0.07663	0.07502	0.0702	0.06982	0.07061	0.07168	0.06982	0.06982	0.07208	0.07057	0.06982	0.06982	0.07384
CHD117B	0.10393	0.10393	0.09577	0.09655	0.10468	0.10698	0.07424	0.07458	0.07424	0.07424	0.0735	0.07286	0.07424	0.0709	0.07053	0.07201	0.07308	0.07053	0.07053	0.07273	0.07053	0.07053	0.07053	0.07527
CHD122B	0.10393	0.10393	0.09577	0.09655	0.10468	0.10698	0.07424	0.07458	0.07424	0.07424	0.0735	0.07286	0.07424	0.0709	0.07053	0.07201	0.07308	0.07053	0.07053	0.07273	0.07053	0.07053	0.07053	0.07527
KEM111	0.10393	0.10393	0.09577	0.09655	0.10468	0.10698	0.07424	0.07458	0.07424	0.07424	0.0735	0.07286	0.07424	0.0709	0.07053	0.07201	0.07308	0.07053	0.07053	0.07273	0.07053	0.07053	0.07053	0.07527
KEM115	0.10393	0.10393	0.09577	0.09655	0.10468	0.10698	0.07424	0.07458	0.07424	0.07424	0.0735	0.07286	0.07424	0.0709	0.07053	0.07201	0.07308	0.07053	0.07053	0.07273	0.07053	0.07053	0.07053	0.07527
KEM112	0.10393	0.10393	0.09577	0.09655	0.10468	0.10698	0.07424	0.07458	0.07424	0.07424	0.0735	0.07286	0.07424	0.0709	0.07053	0.07201	0.07308	0.07053	0.07053	0.07273	0.07053	0.07053	0.07053	0.07527
KEM113	0.10393	0.10393	0.09577	0.09655	0.10468	0.10698	0.07424	0.07458	0.07424	0.07424	0.0735	0.07286	0.07424	0.0709	0.07053	0.07201	0.07308	0.07053	0.07053	0.07273	0.07053	0.07053	0.07053	0.07527
LEN41	0.10448	0.10448	0.09628	0.0963	0.10522	0.10757	0.07458	0.07465	0.07458	0.07458	0.07383	0.0732	0.07458	0.07095	0.07089	0.07203	0.07194	0.07089	0.07089	0.07426	0.07234	0.07235	0.07235	0.07686
GIL57B	0.10542	0.10542	0.09725	0.09654	0.10616	0.10846	0.07572	0.07606	0.07572	0.07572	0.07498	0.07434	0.07572	0.0724	0.07275	0.07352	0.07382	0.07275	0.07275	0.07499	0.0735	0.0735	0.0735	0.07825
CHE140D	0.10842	0.10841	0.10025	0.09954	0.10767	0.107	0.0765	0.07683	0.0765	0.0765	0.07575	0.07512	0.0765	0.07317	0.07353	0.07354	0.07387	0.07353	0.07353	0.07424	0.07353	0.07353	0.07353	0.07827
GIL88	0.10171	0.10171	0.09503	0.09581	0.10468	0.10029	0.0735	0.07383	0.0735	0.0735	0.07275	0.07212	0.07201	0.06193	0.06162	0.06291	0.06405	0.06162	0.06162	0.06438	0.06236	0.0631	0.0631	0.06558
MER10	0.11284	0.11284	0.10765	0.10842	0.11284	0.11513	0.08315	0.08348	0.08315	0.08315	0.08241	0.08175	0.08166	0.07458	0.07424	0.07493	0.07605	0.07424	0.07424	0.07871	0.07869	0.07647	0.07647	0.07895

Appendix C (continued)

	RAM47B	SEM146	SEM152E	SEM160C	LAB53	MesspG	NIP66	GIL56	FUS2	FUS3	BAT39	GIL38	SEM159D	LAB81	LAY55	LAB83	LAB79	LAB74	LAB76	LAY86	MER19	CHE140F	CHE5	MesspD
MER17	0.11359	0.11359	0.10839	0.10917	0.11284	0.11662	0.08166	0.08199	0.08166	0.08166	0.08092	0.08026	0.08018	0.07457	0.07424	0.07494	0.07606	0.07424	0.07424	0.07872	0.07869	0.07647	0.07647	0.07895
SEM162	0.11037	0.11037	0.10515	0.10593	0.10813	0.11418	0.07977	0.07979	0.07977	0.07977	0.07903	0.07837	0.07977	0.0739	0.07383	0.07427	0.07568	0.07383	0.07383	0.07652	0.0768	0.07457	0.07457	0.0768
SEM165	0.10987	0.10987	0.10468	0.10545	0.10765	0.11365	0.07944	0.07976	0.07944	0.07944	0.07869	0.07803	0.07944	0.07383	0.0735	0.07421	0.07533	0.0735	0.0735	0.07648	0.07647	0.07424	0.07424	0.07672
SEM163	0.10987	0.10987	0.10468	0.10545	0.10765	0.11365	0.07944	0.07976	0.07944	0.07944	0.07869	0.07803	0.07944	0.07383	0.0735	0.07421	0.07533	0.0735	0.0735	0.07648	0.07647	0.07424	0.07424	0.07672
SEM164	0.10987	0.10987	0.10468	0.10545	0.10765	0.11365	0.07944	0.07976	0.07944	0.07944	0.07869	0.07803	0.07944	0.07383	0.0735	0.07421	0.07533	0.0735	0.0735	0.07648	0.07647	0.07424	0.07424	0.07672
SEM166	0.10987	0.10987	0.10468	0.10545	0.10765	0.11365	0.07944	0.07976	0.07944	0.07944	0.07869	0.07803	0.07944	0.07383	0.0735	0.07421	0.07533	0.0735	0.0735	0.07648	0.07647	0.07424	0.07424	0.07672
DAN78	0.11081	0.11007	0.10394	0.10474	0.10928	0.11015	0.08483	0.08519	0.08483	0.08483	0.08406	0.08341	0.08333	0.07145	0.07112	0.0714	0.07258	0.07112	0.07112	0.07453	0.07493	0.07569	0.07569	0.07518
DAN77	0.11004	0.11004	0.10484	0.10561	0.11004	0.11011	0.08549	0.08583	0.08549	0.08549	0.08475	0.0841	0.08401	0.07169	0.07138	0.07193	0.07308	0.07138	0.07138	0.07495	0.07509	0.07584	0.07584	0.07534
TKA109	0.10987	0.10987	0.10468	0.10545	0.10987	0.10994	0.08537	0.08571	0.08537	0.08537	0.08463	0.08398	0.08389	0.07158	0.07127	0.07191	0.07306	0.07127	0.07127	0.07494	0.07498	0.07572	0.07572	0.07523
TKA110	0.10987	0.10987	0.10468	0.10545	0.10987	0.10994	0.08537	0.08571	0.08537	0.08537	0.08463	0.08398	0.08389	0.07158	0.07127	0.07191	0.07306	0.07127	0.07127	0.07494	0.07498	0.07572	0.07572	0.07523
TKS130	0.10987	0.10987	0.10468	0.10545	0.10987	0.10994	0.08537	0.08571	0.08537	0.08537	0.08463	0.08398	0.08389	0.07158	0.07127	0.07191	0.07306	0.07127	0.07127	0.07494	0.07498	0.07572	0.07572	0.07523
TKS129	0.11136	0.11136	0.10616	0.10694	0.11136	0.10994	0.08686	0.08721	0.08686	0.08686	0.08612	0.08547	0.08537	0.07159	0.07127	0.07192	0.07306	0.07127	0.07127	0.07646	0.07647	0.07721	0.07721	0.07672
TKS131	0.11062	0.11062	0.10542	0.10619	0.11062	0.10919	0.08612	0.08646	0.08612	0.08612	0.08537	0.08473	0.08463	0.07084	0.07053	0.07115	0.07231	0.07053	0.07053	0.0757	0.07572	0.07647	0.07647	0.07597
BAT43	0.11062	0.11062	0.10542	0.10619	0.11062	0.10919	0.08612	0.08646	0.08612	0.08612	0.08537	0.08473	0.08463	0.07084	0.07053	0.07115	0.07231	0.07053	0.07053	0.0757	0.07572	0.07647	0.07647	0.07597
POR96	0.1079	0.10789	0.1027	0.10347	0.1079	0.11022	0.08184	0.08218	0.08184	0.08184	0.0811	0.08044	0.08036	0.06879	0.06849	0.06907	0.07024	0.06849	0.06849	0.07508	0.07512	0.07438	0.07438	0.07538
TKR133	0.10913	0.10913	0.10542	0.1062	0.10913	0.11142	0.08241	0.08274	0.08241	0.08241	0.08166	0.08101	0.08092	0.07158	0.07127	0.07193	0.07308	0.07127	0.07127	0.07344	0.0735	0.07275	0.07275	0.07375
TKS127B	0.10913	0.10913	0.10542	0.1062	0.10913	0.11142	0.08241	0.08274	0.08241	0.08241	0.08166	0.08101	0.08092	0.07158	0.07127	0.07193	0.07308	0.07127	0.07127	0.07344	0.0735	0.07275	0.07275	0.07375
BAT7A	0.10913	0.10913	0.10542	0.10619	0.11062	0.11365	0.08241	0.08274	0.08241	0.08241	0.08166	0.08101	0.08092	0.07159	0.07127	0.07191	0.07306	0.07127	0.07127	0.07417	0.07424	0.0735	0.0735	0.07449
POR32	0.10908	0.10909	0.10386	0.10464	0.10909	0.11216	0.07992	0.07994	0.07992	0.07992	0.07917	0.07852	0.07842	0.07102	0.07098	0.07124	0.07195	0.07098	0.07098	0.07348	0.07396	0.07321	0.07321	0.07396
MER18	0.10839	0.10839	0.10319	0.10397	0.10839	0.11142	0.07944	0.07976	0.07944	0.07944	0.07869	0.07804	0.07795	0.07085	0.07053	0.07116	0.07231	0.07053	0.07053	0.07343	0.0735	0.07275	0.07275	0.07375
MER11	0.10833	0.10834	0.10311	0.10312	0.10834	0.11291	0.07917	0.0792	0.07917	0.07917	0.07842	0.07777	0.07768	0.07176	0.07172	0.072	0.07195	0.07172	0.07172	0.07424	0.0747	0.07395	0.07395	0.0747
MER71	0.10839	0.10839	0.10319	0.10397	0.10839	0.11142	0.07944	0.07976	0.07944	0.07944	0.07869	0.07804	0.07795	0.07085	0.07053	0.07116	0.07231	0.07053	0.07053	0.07343	0.0735	0.07275	0.07275	0.07375
PTL35	0.10839	0.10839	0.10319	0.10397	0.10839	0.11142	0.07944	0.07976	0.07944	0.07944	0.07869	0.07804	0.07795	0.07085	0.07053	0.07116	0.07231	0.07053	0.07053	0.07343	0.0735	0.07275	0.07275	0.07375
MER16	0.1093	0.1093	0.10406	0.10484	0.1093	0.11239	0.07984	0.07987	0.07984	0.07984	0.07908	0.07843	0.07834	0.07093	0.07086	0.0711	0.07252	0.07086	0.07086	0.07339	0.07386	0.07311	0.07311	0.07388
MER13	0.10795	0.10795	0.10274	0.10352	0.10796	0.111	0.07892	0.07925	0.07892	0.07892	0.07817	0.07751	0.07742	0.07031	0.06999	0.07054	0.07169	0.06999	0.06999	0.07355	0.07371	0.07297	0.07297	0.07396
POR31	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07085	0.07053	0.07117	0.07231	0.07053	0.07053	0.07646	0.07647	0.07572	0.07572	0.07674
CHE140C	0.10675	0.10675	0.10152	0.10154	0.10675	0.11131	0.07837	0.0784	0.07837	0.07837	0.07762	0.07697	0.07687	0.07095	0.07092	0.07126	0.0712	0.07092	0.07092	0.07651	0.07688	0.07613	0.07613	0.07689
POR168	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07085	0.07053	0.07117	0.07231	0.07053	0.07053	0.07646	0.07647	0.07572	0.07572	0.07674
CHE139A	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07085	0.07053	0.07117	0.07231	0.07053	0.07053	0.07646	0.07647	0.07572	0.07572	0.07674
SEM147	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
SEM161A	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
KUN148	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
KUN149	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
SEM151	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
SEM152A	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
SEM156	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
SEM144C	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
SEM158A	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07086	0.07053	0.07118	0.07231	0.07053	0.07053	0.07723	0.07721	0.07647	0.07647	0.07748
SEM160A	0.1069	0.1069	0.10171	0.10248	0.1069	0.11142	0.07869	0.07903	0.07869	0.07869	0.07795	0.0773	0.07721	0.07161	0.07127	0.07194	0.07307	0.07127	0.07127	0.07798	0.07795	0.07721	0.07721	0.07822
MER23	0.10701	0.10701	0.10176	0.10254	0.10629	0.11088	0.07757	0.0779	0.07757	0.07757	0.07681	0.07616	0.07606	0.07119	0.07085	0.07114	0.07229	0.07085	0.07085	0.0765	0.07685	0.07611	0.07611	0.07716
CHE3	0.107	0.107	0.1017	0.10249	0.10628	0.11092	0.0774	0.07743	0.0774	0.0774	0.07664	0.07598	0.07589	0.07067	0.07062	0.07063	0.07133	0.07062	0.07062	0.07598	0.07667	0.07592	0.07592	0.07669
MER22	0.10706	0.10706	0.10186	0.10264	0.10706	0.11159	0.0788	0.07913	0.0788	0.0788	0.07805	0.0774	0.07731	0.07171	0.07137	0.07195	0.07308	0.07137	0.07137	0.07724	0.07732	0.07657	0.07657	0.07759
CHE142	0.10616	0.10616	0.10097	0.10174	0.10616	0.11068	0.07795	0.07828	0.07795	0.07795	0.07721	0.07655	0.07647	0.07085	0.07053	0.07117	0.07231	0.07053	0.07053	0.07646	0.07647	0.07572	0.07572	0.07674
CHE138E	0.10952	0.10879	0.10409	0.10491	0.10952	0.11201	0.06533	0.06564	0.06533	0.06533	0.06455	0.06387	0.06999	0.07032	0.06996	0.07032	0.07149	0.06996	0.06996	0.07746	0.07774	0.07697	0.07697	0.07808
Hap.saxi	0.12175	0.12175	0.11507	0.11512	0.11284	0.11588	0.10616	0.1066	0.10616	0.10616	0.10542	0.10483	0.10319	0.10519	0.10468	0.10605	0.10697	0.10468	0.10468	0.10749	0.10839	0.10468	0.10468	0.10877
LAY54	0.12935	0.13086	0.12562	0.1264	0.12484	0.12866	0.12257	0.12303	0.12257	0.12257	0.12332	0.12203	0.12558	0.11862	0.11808	0.11872	0.11963	0.11808	0.11808	0.13531	0.1324	0.13616	0.13616	0.13735
Nem.ting	0.17287	0.17284	0.17032	0.17031	0.16435	0.17046	0.17201	0.17202	0.17201	0.17201	0.17284	0.17059	0.17117	0.16791	0.16789	0.16777	0.16774	0.16789	0.16789	0.16966	0.16801	0.16804	0.16804	0.16456
Heri.fluv	0.16571	0.16496	0.16195	0.16275	0.159	0.15389	0.16421	0.16506	0.16421	0.16421	0.16345	0.1644	0.1642	0.16298	0.16198	0.16302	0.1634	0.16198	0.16198	0.16835	0.16574	0.16424	0.16424	0.16346
Pse.roscf	0.17479	0.17643	0.18054	0.18059	0.17234	0.16749	0.17409	0.1741	0.17409	0.17409	0.17491	0.17354	0.17325	0.17238	0.17236	0.17222	0.17218	0.17236	0.17236	0.16825	0.16668	0.16834	0.16834	0.16497
MER91	0.17966	0.18263	0.17817	0.17897	0.17372	0.17012	0.17446	0.17459	0.17446	0.17446	0.1752	0.1739	0.17075	0.17031	0.17001	0.17157	0.17263	0.17001	0.17001	0.17297	0.17149	0.17149	0.17149	0.16924

Appendix C (continued)

	RAM47B	SEM146	SEM152E	SEM160C	LAB53	MesspG	NIP66	GIL56	FUS2	FUS3	BAT39	GIL38	SEM159D	LAB81	LAY55	LAB83	LAB79	LAB74	LAB76	LAY86	MER19	CHE140F	CHE5	MesspD
MER95	0.17966	0.18263	0.17817	0.17897	0.17372	0.17012	0.17446	0.17459	0.17446	0.17446	0.1752	0.1739	0.17075	0.17031	0.17001	0.17157	0.17263	0.17001	0.17001	0.17297	0.17149	0.17149	0.17149	0.16924
CHE6	0.17966	0.18263	0.17817	0.17897	0.17372	0.17012	0.17446	0.17459	0.17446	0.17446	0.1752	0.1739	0.17075	0.17031	0.17001	0.17157	0.17263	0.17001	0.17001	0.17297	0.17149	0.17149	0.17149	0.16924
CHE7	0.17966	0.18263	0.17817	0.17897	0.17372	0.17012	0.17446	0.17459	0.17446	0.17446	0.1752	0.1739	0.17075	0.17031	0.17001	0.17157	0.17263	0.17001	0.17001	0.17297	0.17149	0.17149	0.17149	0.16924
POR100	0.17966	0.18263	0.17817	0.17897	0.17372	0.17012	0.17446	0.17459	0.17446	0.17446	0.1752	0.1739	0.17075	0.17031	0.17001	0.17157	0.17263	0.17001	0.17001	0.17297	0.17149	0.17149	0.17149	0.16924
MER21	0.17892	0.1804	0.17595	0.17674	0.17149	0.16788	0.17223	0.17317	0.17223	0.17223	0.17298	0.17166	0.16852	0.16886	0.16778	0.1701	0.17036	0.16778	0.16778	0.17149	0.16927	0.16927	0.16927	0.16779
POR27	0.17966	0.18114	0.17669	0.17748	0.17223	0.16863	0.17298	0.17391	0.17298	0.17298	0.17372	0.1724	0.16927	0.16961	0.16852	0.17085	0.17111	0.16852	0.16852	0.17225	0.17001	0.17001	0.17001	0.16853
CHE4	0.17896	0.18044	0.17599	0.17678	0.17153	0.16792	0.17227	0.1732	0.17227	0.17227	0.17301	0.17169	0.16856	0.1689	0.16782	0.17014	0.17039	0.16782	0.16782	0.17154	0.16931	0.16931	0.16931	0.16783
Por.fluv	0.18291	0.18319	0.17884	0.17996	0.17729	0.15996	0.15559	0.15665	0.15559	0.15559	0.15664	0.15377	0.15633	0.13669	0.13556	0.13679	0.1376	0.13556	0.13556	0.16075	0.15929	0.15718	0.15718	0.15813
Petro.mac	0.17821	0.1798	0.17977	0.1806	0.17744	0.1785	0.1697	0.16974	0.1697	0.1697	0.16892	0.16915	0.17126	0.16678	0.1666	0.16669	0.16795	0.1666	0.1666	0.17538	0.17517	0.17672	0.17672	0.17589
Dipsimplx	0.18923	0.1893	0.18565	0.18661	0.18764	0.18118	0.19387	0.194	0.19387	0.19387	0.19476	0.19329	0.19303	0.18436	0.18402	0.18437	0.18562	0.18402	0.18402	0.19332	0.19203	0.19114	0.19114	0.18836
DipspB	0.20193	0.20193	0.1997	0.20052	0.19822	0.19911	0.20787	0.2089	0.20787	0.20787	0.20861	0.20734	0.20713	0.20167	0.20045	0.1964	0.19652	0.20045	0.20045	0.20683	0.2101	0.20935	0.20935	0.20797
DipspC	0.17892	0.17966	0.17892	0.17971	0.17595	0.17757	0.1804	0.1813	0.1804	0.1804	0.18114	0.17985	0.17966	0.17628	0.1752	0.17684	0.1771	0.1752	0.1752	0.18811	0.1856	0.18486	0.18486	0.18338
POR169	0.19311	0.19385	0.19154	0.19159	0.19007	0.19096	0.18864	0.18864	0.18864	0.18864	0.18941	0.1881	0.19018	0.18477	0.18471	0.18471	0.18462	0.18471	0.18471	0.20586	0.20335	0.20412	0.20412	0.20174
TKS126	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
POR26	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
CHD121	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
CHD123	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
POR29	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
POR24	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
CHD118	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
TKS128	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
POR33	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18392	0.18404	0.18263	0.18263	0.20502	0.20119	0.20193	0.20193	0.20059
POR94	0.19005	0.19005	0.18782	0.18862	0.18708	0.18723	0.1856	0.18661	0.1856	0.1856	0.18634	0.18506	0.18708	0.18307	0.18189	0.18316	0.18329	0.18189	0.18189	0.20426	0.20045	0.20119	0.20119	0.19985
MER8	0.19005	0.19005	0.18782	0.18862	0.18708	0.18723	0.1856	0.18661	0.1856	0.1856	0.18634	0.18506	0.18708	0.18307	0.18189	0.18316	0.18329	0.18189	0.18189	0.20426	0.20045	0.20119	0.20119	0.19985
GIL42	0.19079	0.19079	0.18857	0.18936	0.18782	0.18798	0.18634	0.18735	0.18634	0.18634	0.18708	0.1858	0.18782	0.18382	0.18263	0.18391	0.18404	0.18263	0.18263	0.20501	0.20119	0.20193	0.20193	0.20059
SEM155	0.19079	0.19079	0.18857	0.18936	0.18782	0.18649	0.18486	0.18586	0.18486	0.18486	0.1856	0.18431	0.18634	0.18233	0.18114	0.1824	0.18253	0.18114	0.18114	0.2035	0.1997	0.20045	0.20045	0.1991
LAB46	0.18409	0.1841	0.18559	0.1849	0.1826	0.18278	0.18931	0.19027	0.18931	0.18931	0.19006	0.18881	0.18782	0.18592	0.18561	0.18586	0.1854	0.18561	0.18561	0.19706	0.19679	0.19381	0.19381	0.19388
RAM65	0.18409	0.1841	0.18559	0.1849	0.1826	0.18278	0.18931	0.19027	0.18931	0.18931	0.19006	0.18881	0.18782	0.18592	0.18561	0.18586	0.1854	0.18561	0.18561	0.19706	0.19679	0.19381	0.19381	0.19388
LAB80	0.1826	0.18261	0.1841	0.18489	0.1811	0.18129	0.18782	0.18877	0.18782	0.18782	0.18856	0.18731	0.18633	0.18524	0.18412	0.18516	0.1854	0.18412	0.18412	0.19637	0.19529	0.19231	0.19231	0.19239
RAM47A	0.18227	0.18227	0.18376	0.18455	0.18078	0.18091	0.18748	0.18842	0.18748	0.18748	0.18822	0.18697	0.18597	0.18486	0.18375	0.1851	0.18533	0.18375	0.18375	0.1963	0.19497	0.19198	0.19198	0.19206
LAB82	0.18206	0.18206	0.18356	0.18435	0.18057	0.18073	0.18728	0.18822	0.18728	0.18728	0.18802	0.18677	0.18578	0.18468	0.18356	0.18513	0.18537	0.18356	0.18356	0.19633	0.19476	0.19177	0.19177	0.19185
LAB84	0.18168	0.18169	0.18318	0.18397	0.18019	0.18033	0.1869	0.18784	0.1869	0.1869	0.18765	0.18639	0.18539	0.18428	0.18317	0.1851	0.18533	0.18317	0.18317	0.1963	0.19441	0.19142	0.19142	0.19149
LAB75	0.18111	0.18111	0.18261	0.1834	0.17961	0.17979	0.18707	0.18803	0.18707	0.18707	0.18782	0.18657	0.18558	0.18523	0.18411	0.18517	0.1854	0.18411	0.18411	0.19486	0.1938	0.19082	0.19082	0.19089
CHE143C	0.19045	0.19314	0.19128	0.19129	0.18516	0.19595	0.19569	0.19559	0.19569	0.19569	0.19479	0.19506	0.19569	0.18679	0.18681	0.18759	0.18759	0.18681	0.18681	0.2029	0.20103	0.20019	0.20019	0.20187
CHE141	0.18189	0.18411	0.18263	0.18342	0.17743	0.188	0.19376	0.19472	0.19376	0.19376	0.19302	0.19245	0.19376	0.18077	0.17966	0.18147	0.18168	0.17966	0.17966	0.20184	0.1997	0.19822	0.19822	0.20051
CHE139B	0.18189	0.18411	0.18263	0.18342	0.17743	0.188	0.19376	0.19472	0.19376	0.19376	0.19302	0.19245	0.19376	0.18077	0.17966	0.18147	0.18168	0.17966	0.17966	0.20184	0.1997	0.19822	0.19822	0.20051
SAY134	0.18189	0.18411	0.18263	0.18342	0.17743	0.188	0.19376	0.19472	0.19376	0.19376	0.19302	0.19245	0.19376	0.18077	0.17966	0.18147	0.18168	0.17966	0.17966	0.20184	0.1997	0.19822	0.19822	0.20051
SAY135	0.18189	0.18411	0.18263	0.18342	0.17743	0.188	0.19376	0.19472	0.19376	0.19376	0.19302	0.19245	0.19376	0.18077	0.17966	0.18147	0.18168	0.17966	0.17966	0.20184	0.1997	0.19822	0.19822	0.20051
CHE137	0.18189	0.18411	0.18263	0.18342	0.17743	0.188	0.19376	0.19472	0.19376	0.19376	0.19302	0.19245	0.19376	0.18077	0.17966	0.18147	0.18168	0.17966	0.17966	0.20184	0.1997	0.19822	0.19822	0.20051
SEM144E	0.17446	0.17446	0.17446	0.17526	0.17595	0.17609	0.16927	0.17013	0.16927	0.16927	0.16852	0.16871	0.1663	0.16063	0.15961	0.16252	0.16283	0.15961	0.15961	0.17606	0.17223	0.17149	0.17149	0.1722
POR99	0.17446	0.17446	0.17446	0.17526	0.17595	0.17609	0.16927	0.17013	0.16927	0.16927	0.16852	0.16871	0.1663	0.16063	0.15961	0.16252	0.16283	0.15961	0.15961	0.17606	0.17223	0.17149	0.17149	0.1722
POR167	0.17446	0.17446	0.17446	0.17526	0.17595	0.17609	0.16927	0.17013	0.16927	0.16927	0.16852	0.16871	0.1663	0.16063	0.15961	0.16252	0.16283	0.15961	0.15961	0.17606	0.17223	0.17149	0.17149	0.1722
MER64	0.17223	0.17223	0.17223	0.17303	0.17372	0.17387	0.16704	0.16789	0.16704	0.16704	0.1663	0.16648	0.16407	0.15839	0.15739	0.16024	0.16057	0.15739	0.15739	0.17378	0.17001	0.16927	0.16927	0.16997
LAY52	0.17298	0.17298	0.17298	0.17377	0.17446	0.17461	0.16778	0.16865	0.16778	0.16778	0.16704	0.16723	0.16481	0.15914	0.15813	0.16102	0.16133	0.15813	0.15813	0.17304	0.16927	0.16852	0.16852	0.16923
GIL67	0.17473	0.17474	0.17474	0.17554	0.17703	0.1772	0.16865	0.16953	0.16865	0.16865	0.16789	0.16808	0.16562	0.16071	0.15966	0.16084	0.16116	0.15966	0.15966	0.17282	0.17095	0.17021	0.17021	0.17094
LAY51	0.17298	0.17298	0.17298	0.17377	0.17446	0.17461	0.16778	0.16865	0.16778	0.16778	0.16704	0.16723	0.16481	0.15914	0.15813	0.16102	0.16133	0.15813	0.15813	0.17304	0.16927	0.16852	0.16852	0.16923
GIL48B	0.17298	0.17298	0.17298	0.17377	0.17446	0.17461	0.16778	0.16865	0.16778	0.16778	0.16704	0.16723	0.16481	0.15914	0.15813	0.16102	0.16133	0.15813	0.15813	0.17304	0.16927	0.16852	0.16852	0.16923
CHE2	0.17075	0.17075	0.17075	0.17154	0.17223	0.17238	0.16555	0.1664	0.16555	0.16555	0.16481	0.16499	0.16258	0.1569	0.1559	0.15873	0.15906	0.1559	0.1559	0.17226	0.16852	0.16778	0.16778	0.16848

Appendix C (continued)

	POR34	CHE143A	CHE138C	TKS127A	MesspC	CHD117A	CHD122A	CHD120	KU1065	BIS62	BIS63	SEM159A	SEM161B	MER14	MER15	CHE1	CHD117B	CHD122B	KEM111	KEM115	KEM112	KEM113	LEN41	GIL57B
POR34	N																							
CHE143A	0	N																						
CHE138C	0	0	N																					
TKS127A	0.00074	0.00074	0.00074	N																				
MesspC	0.01411	0.01411	0.01411	0.01485	N																			
CHD117A	0.0631	0.0631	0.0631	0.06385	0.06236	N																		
CHD122A	0.0631	0.0631	0.0631	0.06385	0.06236	0	N																	
CHD120	0.0631	0.0631	0.0631	0.06385	0.06236	0	0	N																
KU1065	0.0631	0.0631	0.0631	0.06385	0.06236	0.00148	0.00148	0.00148	N															
BIS62	0.06339	0.06339	0.06339	0.06414	0.06114	0.02387	0.02387	0.02387	0.02387	N														
BIS63	0.06385	0.06385	0.06385	0.06459	0.06236	0.02301	0.02301	0.02301	0.02301	0.00074	N													
SEM159A	0.05865	0.05865	0.05865	0.05939	0.05939	0.02301	0.02301	0.02301	0.0245	0.02238	0.02153	N												
SEM161B	0.05665	0.05665	0.05665	0.05739	0.05739	0.02233	0.02233	0.02233	0.02382	0.02168	0.02083	0	N											
MER14	0.06385	0.06385	0.06385	0.06459	0.0631	0.02821	0.02821	0.02821	0.02821	0.02539	0.0245	0.02598	0.02534	N										
MER15	0.06236	0.06236	0.06236	0.0631	0.06162	0.02673	0.02673	0.02673	0.02673	0.02389	0.02301	0.0245	0.02385	0.00148	N									
CHE1	0.05793	0.05793	0.05793	0.05867	0.0557	0.02897	0.02897	0.02897	0.03046	0.0321	0.0312	0.02971	0.02906	0.02304	0.02155	N								
CHD117B	0.06682	0.06682	0.06682	0.06756	0.06459	0.06459	0.06459	0.06459	0.06607	0.05669	0.05716	0.05568	0.05286	0.05494	0.05419	0.05571	N							
CHD122B	0.06682	0.06682	0.06682	0.06756	0.06459	0.06459	0.06459	0.06459	0.06607	0.05669	0.05716	0.05568	0.05286	0.05494	0.05419	0.05571	0	N						
KEM111	0.06682	0.06682	0.06682	0.06756	0.06459	0.06459	0.06459	0.06459	0.06607	0.05669	0.05716	0.05568	0.05286	0.05494	0.05419	0.05571	0	0	N					
KEM115	0.06682	0.06682	0.06682	0.06756	0.06459	0.06459	0.06459	0.06459	0.06607	0.05669	0.05716	0.05568	0.05286	0.05494	0.05419	0.05571	0	0	0	N				
KEM112	0.06682	0.06682	0.06682	0.06756	0.06459	0.06459	0.06459	0.06459	0.06607	0.05669	0.05716	0.05568	0.05286	0.05494	0.05419	0.05571	0	0	0	0	N			
KEM113	0.06682	0.06682	0.06682	0.06756	0.06459	0.06459	0.06459	0.06459	0.06607	0.05669	0.05716	0.05568	0.05286	0.05494	0.05419	0.05571	0	0	0	0	0	N		
LEN41	0.06713	0.06713	0.06713	0.06788	0.06564	0.06492	0.06492	0.06492	0.06641	0.05825	0.05895	0.05746	0.05463	0.05674	0.056	0.05751	0.00224	0.00224	0.00224	0.00224	0.00224	0.00224	N	
GIL57B	0.06904	0.06904	0.06904	0.0683	0.06682	0.06756	0.06756	0.06756	0.06904	0.05894	0.06013	0.05865	0.05584	0.05791	0.05716	0.05868	0.00297	0.00297	0.00297	0.00297	0.00297	0.00297	0.00448	N
CHE140D	0.07129	0.07129	0.07129	0.07204	0.07055	0.0676	0.0676	0.0676	0.06909	0.06046	0.06166	0.06917	0.05737	0.05796	0.05721	0.06021	0.00966	0.00966	0.00966	0.00966	0.00966	0.00966	0.0097	0.01114
GIL88	0.05568	0.05568	0.05568	0.05642	0.05791	0.05122	0.05122	0.05122	0.05271	0.04997	0.05048	0.04603	0.04318	0.04751	0.04677	0.05274	0.03489	0.03489	0.03489	0.03489	0.03489	0.03489	0.03655	0.03786
MER10	0.07944	0.07944	0.07944	0.07944	0.08166	0.07647	0.07647	0.07647	0.07647	0.08128	0.08018	0.07275	0.07	0.07127	0.07053	0.0765	0.07127	0.07127	0.07127	0.07127	0.07127	0.07127	0.07307	0.0735
MER17	0.07944	0.07944	0.07944	0.07944	0.08166	0.07647	0.07647	0.07647	0.07647	0.08053	0.07944	0.07275	0.07001	0.07127	0.07053	0.0765	0.07053	0.07053	0.07053	0.07053	0.07053	0.07053	0.07233	0.07275
SEM162	0.07606	0.07606	0.07606	0.07606	0.07753	0.07609	0.07609	0.07609	0.07609	0.07838	0.07758	0.07236	0.06962	0.06941	0.06866	0.07466	0.06714	0.06714	0.06714	0.06714	0.06714	0.06714	0.06867	0.06938
SEM165	0.07572	0.07572	0.07572	0.07572	0.07795	0.07572	0.07572	0.07572	0.07572	0.07829	0.07721	0.07201	0.06928	0.06904	0.0683	0.07427	0.06682	0.06682	0.06682	0.06682	0.06682	0.06682	0.0686	0.06904
SEM163	0.07572	0.07572	0.07572	0.07572	0.07795	0.07572	0.07572	0.07572	0.07572	0.07829	0.07721	0.07201	0.06928	0.06904	0.0683	0.07427	0.06682	0.06682	0.06682	0.06682	0.06682	0.06682	0.0686	0.06904
SEM164	0.07572	0.07572	0.07572	0.07572	0.07795	0.07572	0.07572	0.07572	0.07572	0.07829	0.07721	0.07201	0.06928	0.06904	0.0683	0.07427	0.06682	0.06682	0.06682	0.06682	0.06682	0.06682	0.0686	0.06904
SEM166	0.07572	0.07572	0.07572	0.07572	0.07795	0.07572	0.07572	0.07572	0.07572	0.07829	0.07721	0.07201	0.06928	0.06904	0.0683	0.07427	0.06682	0.06682	0.06682	0.06682	0.06682	0.06682	0.0686	0.06904
DAN78	0.07801	0.07801	0.07801	0.07797	0.08489	0.0803	0.0803	0.0803	0.0803	0.08297	0.08183	0.07419	0.07138	0.07184	0.07108	0.07649	0.0696	0.0696	0.0696	0.0696	0.0696	0.0696	0.07147	0.07187
DAN77	0.07732	0.07732	0.07732	0.07732	0.08402	0.07882	0.07882	0.07882	0.07882	0.08141	0.08031	0.07287	0.07014	0.07065	0.06991	0.07514	0.0696	0.0696	0.0696	0.0696	0.0696	0.0696	0.07169	0.07213
TKA109	0.07721	0.07721	0.07721	0.07721	0.08389	0.07869	0.07869	0.07869	0.07869	0.08127	0.08018	0.07275	0.07002	0.07053	0.06978	0.07501	0.06978	0.06978	0.06978	0.06978	0.06978	0.06978	0.07158	0.07201
TKA110	0.07721	0.07721	0.07721	0.07721	0.08389	0.07869	0.07869	0.07869	0.07869	0.08127	0.08018	0.07275	0.07002	0.07053	0.06978	0.07501	0.06978	0.06978	0.06978	0.06978	0.06978	0.06978	0.07158	0.07201
TKS130	0.07721	0.07721	0.07721	0.07721	0.08389	0.07869	0.07869	0.07869	0.07869	0.08127	0.08018	0.07275	0.07002	0.07053	0.06978	0.07501	0.06978	0.06978	0.06978	0.06978	0.06978	0.06978	0.07158	0.07201
TKS129	0.07721	0.07721	0.07721	0.07721	0.08389	0.07869	0.07869	0.07869	0.07869	0.08128	0.08018	0.07275	0.07002	0.07053	0.06978	0.07501	0.06978	0.06978	0.06978	0.06978	0.06978	0.06978	0.07159	0.07201
TKS131	0.07647	0.07647	0.07647	0.07647	0.08315	0.07795	0.07795	0.07795	0.07795	0.08053	0.07944	0.07201	0.06928	0.06978	0.06904	0.07427	0.06904	0.06904	0.06904	0.06904	0.06904	0.06904	0.07084	0.07127
BAT43	0.07647	0.07647	0.07647	0.07647	0.08315	0.07795	0.07795	0.07795	0.07795	0.08053	0.07944	0.07201	0.06928	0.06978	0.06904	0.07427	0.06904	0.06904	0.06904	0.06904	0.06904	0.06904	0.07084	0.07127
POR96	0.07445	0.07445	0.07445	0.07445	0.08037	0.07815	0.07815	0.07815	0.07815	0.07774	0.07814	0.0722	0.06944	0.06995	0.06921	0.0752	0.06699	0.06699	0.06699	0.06699	0.06699	0.06699	0.06878	0.06922
TKR133	0.07498	0.07498	0.07498	0.07498	0.08018	0.07647	0.07647	0.07647	0.07647	0.0783	0.07869	0.07201	0.06928	0.07053	0.06978	0.07576	0.06904	0.06904	0.06904	0.06904	0.06904	0.06904	0.07083	0.07127
TKS127B	0.07498	0.07498	0.07498	0.07498	0.08018	0.07647	0.07647	0.07647	0.07647	0.0783	0.07869	0.07201	0.06928	0.07053	0.06978	0.07576	0.06904	0.06904	0.06904	0.06904	0.06904	0.06904	0.07083	0.07127
BAT7A	0.07647	0.07647	0.07647	0.07647	0.08166	0.08018	0.08018	0.08018	0.08018	0.08128	0.08166	0.07498	0.07225	0.0735	0.07275	0.07948	0.06904	0.06904	0.06904	0.06904	0.06904	0.06904	0.07084	0.07127
POR32	0.07395	0.07395	0.07395	0.07396	0.07844	0.07773	0.07773	0.07773	0.07773	0.07851	0.07922	0.07248	0.06973	0.07104	0.07029	0.07779	0.06801	0.06801	0.06801	0.06801	0.06801	0.06801	0.06954	0.07025
MER18	0.0735	0.0735	0.0735	0.0735	0.07869	0.07721	0.07721	0.07721	0.07721	0.0783	0.07869	0.07201	0.06927	0.07053	0.06978	0.07725	0.06756	0.06756	0.06756	0.06756	0.06756	0.06756	0.06935	0.06978
MER11	0.0747	0.0747	0.0747	0.0747	0.07919	0.07847	0.07847	0.07847	0.07847	0.07923	0.07996	0.07322	0.07047	0.07178	0.07104	0.07853	0.06875	0.06875	0.06875	0.06875	0.06875	0.06875	0.07027	0.07023
MER71	0.0735	0.0735	0.0735	0.0735	0.07869	0.07721	0.07721	0.07721	0.07721	0.0783	0.07869	0.07201	0.06927	0.07053	0.06978	0.07725	0.06756	0.06756	0.06756	0.06756	0.06756	0.06756	0.06935	0.06978
PTL35	0.0735	0.0735	0.0735	0.0735	0.07869	0.07721	0.07721	0.07721	0.07721	0.0783	0.07869	0.07201	0.06927	0.07053	0.06978	0.07725	0.06756	0.06756	0.06756	0.06756	0.06756	0.06756	0.06935	0.06978
MER16	0.07387	0.07387	0.07387	0.07388	0.07841	0.07842	0.07842	0.07842	0.07842	0.07927	0.07997	0.07319	0.07041	0.07165	0.0709	0.07851	0.0679	0.0679	0.0679	0.0679	0.0679	0.0679	0.06945	0.07015

Appendix C (continued)

	POR34	CHE143A	CHE138C	TKS127A	MesspC	CHD117A	CHD122A	CHD120	KU1065	BIS62	BIS63	SEM159A	SEM161B	MER14	MER15	CHE1	CHD117B	CHD122B	KEM111	KEM115	KEM112	KEM113	LEN41	GIL57B
MER13	0.07297	0.07297	0.07297	0.07297	0.07818	0.07669	0.07669	0.07669	0.07669	0.0778	0.07819	0.07149	0.06874	0.06999	0.06925	0.07674	0.06626	0.06626	0.06626	0.06626	0.06626	0.06626	0.06805	0.06849
POR31	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07682	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
CHE140C	0.07165	0.07165	0.07165	0.07165	0.07613	0.07616	0.07616	0.07616	0.07616	0.07692	0.07766	0.07092	0.06816	0.06948	0.06873	0.07622	0.06496	0.06496	0.06496	0.06496	0.06496	0.06496	0.06647	0.06643
POR168	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07682	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
CHE139A	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07682	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
SEM147	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
SEM161A	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
KUN148	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
KUN149	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
SEM151	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
SEM152A	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
SEM156	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
SEM144C	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
SEM158A	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07683	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
SEM160A	0.07201	0.07201	0.07201	0.07201	0.07721	0.07647	0.07647	0.07647	0.07647	0.07608	0.07647	0.06978	0.06702	0.0683	0.06756	0.07502	0.06385	0.06385	0.06385	0.06385	0.06385	0.06385	0.06564	0.06607
MER23	0.07159	0.07159	0.07159	0.07159	0.07689	0.0769	0.0769	0.0769	0.0769	0.07806	0.07845	0.07167	0.06888	0.07012	0.06938	0.07699	0.06486	0.06486	0.06486	0.06486	0.06486	0.06486	0.06666	0.0671
CHE3	0.07136	0.07136	0.07136	0.07132	0.07593	0.07673	0.07673	0.07673	0.07673	0.07754	0.07826	0.07142	0.06861	0.0699	0.06915	0.0768	0.06458	0.06458	0.06458	0.06458	0.06458	0.06458	0.06613	0.06681
MER22	0.07211	0.07211	0.07211	0.07211	0.07658	0.07658	0.07658	0.07658	0.07658	0.07769	0.07807	0.07138	0.06862	0.0699	0.06916	0.07663	0.06543	0.06543	0.06543	0.06543	0.06543	0.06543	0.06723	0.06766
CHE142	0.07127	0.07127	0.07127	0.07127	0.07647	0.07572	0.07572	0.07572	0.07572	0.07682	0.07721	0.07053	0.06777	0.06904	0.0683	0.07576	0.06459	0.06459	0.06459	0.06459	0.06459	0.06459	0.06638	0.06682
CHE138E	0.07618	0.07618	0.07618	0.07611	0.0801	0.0762	0.0762	0.0762	0.0762	0.07509	0.07549	0.0716	0.06873	0.06761	0.06761	0.0724	0.07153	0.07153	0.07153	0.07153	0.07153	0.07153	0.07344	0.07225
Hap.saxi	0.10542	0.10542	0.10542	0.10468	0.10468	0.1069	0.1069	0.1069	0.10616	0.09997	0.10022	0.10468	0.10339	0.10097	0.10097	0.10399	0.09725	0.09725	0.09725	0.09725	0.09725	0.09725	0.09922	0.09725
LAY54	0.12486	0.12486	0.12486	0.12411	0.13163	0.13311	0.13311	0.13311	0.13462	0.1345	0.13463	0.12861	0.12684	0.13458	0.13383	0.13468	0.12712	0.12712	0.12712	0.12712	0.12712	0.12712	0.12922	0.12561
Nem.ting	0.16453	0.16453	0.16453	0.16447	0.16034	0.15609	0.15609	0.15609	0.15524	0.1535	0.15436	0.15947	0.15858	0.15268	0.15437	0.15617	0.15864	0.15864	0.15864	0.15864	0.15864	0.15864	0.15867	0.15857
Heri.fluv	0.16348	0.16348	0.16348	0.16272	0.16048	0.1613	0.1613	0.1613	0.1613	0.15551	0.1553	0.15983	0.1585	0.15378	0.15453	0.16059	0.16276	0.16276	0.16276	0.16276	0.16276	0.16276	0.16451	0.16275
Pse.roscf	0.16661	0.16661	0.16661	0.16657	0.16504	0.16501	0.16501	0.16501	0.16666	0.16501	0.16583	0.165	0.1635	0.15832	0.15832	0.16262	0.17484	0.17484	0.17484	0.17484	0.17484	0.17484	0.17648	0.17472
MER91	0.017298	0.017298	0.017298	0.17298	0.16778	0.1663	0.1663	0.1663	0.16555	0.16656	0.16704	0.17001	0.16864	0.15961	0.15961	0.15969	0.16407	0.16407	0.16407	0.16407	0.16407	0.16407	0.1651	0.16481
MER95	0.017298	0.017298	0.017298	0.17298	0.16778	0.1663	0.1663	0.1663	0.16555	0.16656	0.16704	0.17001	0.16864	0.15961	0.15961	0.15969	0.16407	0.16407	0.16407	0.16407	0.16407	0.16407	0.1651	0.16481
CHE6	0.017298	0.017298	0.017298	0.17298	0.16778	0.1663	0.1663	0.1663	0.16555	0.16656	0.16704	0.17001	0.16864	0.15961	0.15961	0.15969	0.16407	0.16407	0.16407	0.16407	0.16407	0.16407	0.1651	0.16481
CHE7	0.017298	0.017298	0.017298	0.17298	0.16778	0.1663	0.1663	0.1663	0.16555	0.16656	0.16704	0.17001	0.16864	0.15961	0.15961	0.15969	0.16407	0.16407	0.16407	0.16407	0.16407	0.16407	0.1651	0.16481
POR100	0.017298	0.017298	0.017298	0.17298	0.16778	0.1663	0.1663	0.1663	0.16555	0.16656	0.16704	0.17001	0.16864	0.15961	0.15961	0.15969	0.16407	0.16407	0.16407	0.16407	0.16407	0.16407	0.1651	0.16481
MER21	0.17075	0.17075	0.17075	0.17075	0.1663	0.16407	0.16407	0.16407	0.16333	0.16512	0.16481	0.16778	0.1664	0.15739	0.15739	0.15746	0.16184	0.16184	0.16184	0.16184	0.16184	0.16184	0.16365	0.16258
POR27	0.17149	0.17149	0.17149	0.17149	0.1663	0.16481	0.16481	0.16481	0.16407	0.16586	0.16555	0.16852	0.16714	0.15813	0.15813	0.15821	0.16258	0.16258	0.16258	0.16258	0.16258	0.16258	0.16439	0.16333
CHE4	0.17079	0.17079	0.17079	0.17079	0.16634	0.16411	0.16411	0.16411	0.16336	0.16516	0.16485	0.16782	0.16644	0.15743	0.15743	0.15746	0.16188	0.16188	0.16188	0.16188	0.16188	0.16188	0.16368	0.16262
Por.fluv	0.14872	0.14872	0.14872	0.14861	0.14533	0.14471	0.14471	0.14471	0.14471	0.14382	0.14367	0.14914	0.14917	0.14131	0.14024	0.14387	0.15471	0.15471	0.15471	0.15471	0.15471	0.15471	0.15818	0.15571
Petro.mac	0.1667	0.1667	0.1667	0.16664	0.16362	0.16438	0.16438	0.16438	0.16516	0.1615	0.16209	0.159	0.15826	0.1597	0.16048	0.16371	0.15737	0.15737	0.15737	0.15737	0.15737	0.15737	0.15832	0.15808
Dipsimplx	0.1897	0.1897	0.1897	0.1896	0.18245	0.18217	0.18217	0.18217	0.18311	0.17889	0.17949	0.17691	0.17618	0.17497	0.17402	0.17515	0.17699	0.17699	0.17699	0.17699	0.17699	0.17699	0.17911	0.17782
DipspB	0.2049	0.2049	0.2049	0.20416	0.20193	0.20193	0.20193	0.20193	0.20267	0.20093	0.20045	0.18922	0.19774	0.19673	0.19673	0.19681	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.20018	0.20045
DipspC	0.17966	0.17966	0.17966	0.1804	0.17743	0.17743	0.17743	0.17743	0.17817	0.17479	0.17446	0.17223	0.17173	0.17149	0.17149	0.17157	0.17298	0.17298	0.17298	0.17298	0.17298	0.17298	0.17404	0.17446
POR169	0.19633	0.19633	0.19633	0.19711	0.18943	0.19092	0.19092	0.19092	0.19014	0.18626	0.1862	0.18702	0.18645	0.18477	0.18555	0.18546	0.18698	0.18698	0.18698	0.18698	0.18698	0.18698	0.18546	0.18774
TKS126	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
POR26	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
CHD121	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
CHD123	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
POR29	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
POR24	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
CHD118	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
TKS128	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
POR33	0.19451	0.19451	0.19451	0.19525	0.18931	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
POR94	0.19376	0.19376	0.19376	0.19451	0.18857	0.18708	0.18708	0.18708	0.18634	0.18306	0.18189	0.18337	0.18284	0.18189	0.18263	0.18337	0.18486	0.18486	0.18486	0.18486	0.18486	0.18486	0.18458	0.18634
MER8	0.19376	0.19376	0.19376	0.19451	0.18857	0.18708	0.18708	0.18708	0.18634	0.18306	0.18189	0.18337	0.18284	0.18189	0.18263	0.18337	0.18486	0.18486	0.18486	0.18486	0.18486	0.18486	0.18458	0.18634

Appendix C (continued)

	POR34	CHE143A	CHE138C	TKS127A	MesspC	CHD117A	CHD122A	CHD120	KU1065	BIS62	BIS63	SEM159A	SEM161B	MER14	MER15	CHE1	CHD117B	CHD122B	KEM111	KEM115	KEM112	KEM113	LEN41	GIL57B
GIL42	0.19451	0.19451	0.19451	0.19525	0.18782	0.18782	0.18782	0.18782	0.18708	0.18381	0.18263	0.18411	0.18359	0.18263	0.18337	0.18411	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
SEM155	0.19302	0.19302	0.19302	0.19376	0.18782	0.18634	0.18634	0.18634	0.1856	0.18232	0.18114	0.18263	0.1821	0.18114	0.18189	0.18263	0.1856	0.1856	0.1856	0.1856	0.1856	0.1856	0.18532	0.18708
LAB46	0.18708	0.18708	0.18708	0.18783	0.18561	0.18488	0.18488	0.18488	0.18414	0.18144	0.18191	0.18414	0.183	0.17895	0.17969	0.18127	0.18489	0.18489	0.18489	0.18489	0.18489	0.18489	0.18519	0.18489
RAM65	0.18708	0.18708	0.18708	0.18783	0.18561	0.18488	0.18488	0.18488	0.18414	0.18144	0.18191	0.18414	0.183	0.17895	0.17969	0.18127	0.18489	0.18489	0.18489	0.18489	0.18489	0.18489	0.18519	0.18489
LAB80	0.18559	0.18559	0.18559	0.18633	0.18411	0.18339	0.18339	0.18339	0.18264	0.18075	0.18041	0.18263	0.18151	0.17745	0.1782	0.17977	0.18339	0.18339	0.18339	0.18339	0.18339	0.18339	0.18451	0.18488
RAM47A	0.18524	0.18524	0.18524	0.18599	0.18375	0.18301	0.18301	0.18301	0.18226	0.18037	0.18004	0.18229	0.18114	0.17704	0.17778	0.17939	0.18301	0.18301	0.18301	0.18301	0.18301	0.18301	0.18411	0.1845
LAB82	0.18504	0.18504	0.18504	0.18579	0.18356	0.18283	0.18283	0.18283	0.18208	0.18018	0.17985	0.18209	0.18095	0.17687	0.17762	0.17921	0.18283	0.18283	0.18283	0.18283	0.18283	0.18283	0.18394	0.18432
LAB84	0.18466	0.18466	0.18466	0.18541	0.18317	0.18244	0.18244	0.18244	0.18169	0.17979	0.17947	0.18172	0.18058	0.17647	0.17722	0.17882	0.18244	0.18244	0.18244	0.18244	0.18244	0.18244	0.18354	0.18394
LAB75	0.18409	0.18409	0.18409	0.18484	0.18262	0.1819	0.1819	0.1819	0.18115	0.17925	0.17892	0.18115	0.18001	0.17596	0.17671	0.17828	0.1819	0.1819	0.1819	0.1819	0.1819	0.1819	0.18301	0.18339
CHE143C	0.19377	0.19377	0.19377	0.19467	0.18837	0.18862	0.18862	0.18862	0.18772	0.192	0.19293	0.18943	0.18791	0.1884	0.18929	0.18951	0.18251	0.18251	0.18251	0.18251	0.18251	0.18251	0.18061	0.18338
CHE141	0.19451	0.19451	0.19451	0.19525	0.19154	0.1856	0.1856	0.1856	0.18486	0.19121	0.19079	0.18708	0.1859	0.18708	0.18782	0.18865	0.18411	0.18411	0.18411	0.18411	0.18411	0.18411	0.18375	0.1856
CHE139B	0.19451	0.19451	0.19451	0.19525	0.19154	0.1856	0.1856	0.1856	0.18486	0.19121	0.19079	0.18708	0.1859	0.18708	0.18782	0.18865	0.18411	0.18411	0.18411	0.18411	0.18411	0.18411	0.18375	0.1856
SAY134	0.19451	0.19451	0.19451	0.19525	0.19154	0.1856	0.1856	0.1856	0.18486	0.19121	0.19079	0.18708	0.1859	0.18708	0.18782	0.18865	0.18411	0.18411	0.18411	0.18411	0.18411	0.18411	0.18375	0.1856
SAY135	0.19451	0.19451	0.19451	0.19525	0.19154	0.1856	0.1856	0.1856	0.18486	0.19121	0.19079	0.18708	0.1859	0.18708	0.18782	0.18865	0.18411	0.18411	0.18411	0.18411	0.18411	0.18411	0.18375	0.1856
CHE137	0.19451	0.19451	0.19451	0.19525	0.19154	0.1856	0.1856	0.1856	0.18486	0.19121	0.19079	0.18708	0.1859	0.18708	0.18782	0.18865	0.18411	0.18411	0.18411	0.18411	0.18411	0.18411	0.18375	0.1856
SEM144E	0.17372	0.17372	0.17372	0.17298	0.17001	0.16333	0.16333	0.16333	0.16258	0.16434	0.16407	0.16704	0.16574	0.15739	0.15739	0.16638	0.16333	0.16333	0.16333	0.16333	0.16333	0.16333	0.16511	0.16333
POR99	0.17372	0.17372	0.17372	0.17298	0.17001	0.16333	0.16333	0.16333	0.16258	0.16434	0.16407	0.16704	0.16574	0.15739	0.15739	0.16638	0.16333	0.16333	0.16333	0.16333	0.16333	0.16333	0.16511	0.16333
POR167	0.17372	0.17372	0.17372	0.17298	0.17001	0.16333	0.16333	0.16333	0.16258	0.16434	0.16407	0.16704	0.16574	0.15739	0.15739	0.16638	0.16333	0.16333	0.16333	0.16333	0.16333	0.16333	0.16511	0.16333
MER64	0.17149	0.17149	0.17149	0.17075	0.16778	0.16184	0.16184	0.16184	0.1611	0.16284	0.16258	0.16555	0.16426	0.1559	0.1559	0.16489	0.16258	0.16258	0.16258	0.16258	0.16258	0.16258	0.16436	0.16258
LAY52	0.17223	0.17223	0.17223	0.17149	0.16852	0.16184	0.16184	0.16184	0.1611	0.16285	0.16258	0.16555	0.16499	0.1559	0.1559	0.16415	0.16481	0.16481	0.16481	0.16481	0.16481	0.16481	0.1666	0.16481
GIL67	0.17396	0.17396	0.17396	0.17321	0.17022	0.1627	0.1627	0.1627	0.16195	0.16377	0.1635	0.16651	0.16593	0.1567	0.1567	0.16511	0.16645	0.16645	0.16645	0.16645	0.16645	0.16645	0.16827	0.16645
LAY51	0.17223	0.17223	0.17223	0.17149	0.16852	0.16184	0.16184	0.16184	0.1611	0.16285	0.16258	0.16555	0.16499	0.1559	0.1559	0.16415	0.16481	0.16481	0.16481	0.16481	0.16481	0.16481	0.1666	0.16481
GIL48B	0.17223	0.17223	0.17223	0.17149	0.16852	0.16184	0.16184	0.16184	0.1611	0.16285	0.16258	0.16555	0.16499	0.1559	0.1559	0.16415	0.16481	0.16481	0.16481	0.16481	0.16481	0.16481	0.1666	0.16481
CHE2	0.17001	0.17001	0.17001	0.16927	0.1663	0.16036	0.16036	0.16036	0.15961	0.16135	0.1611	0.16407	0.16277	0.15442	0.15442	0.16341	0.1611	0.1611	0.1611	0.1611	0.1611	0.1611	0.16286	0.1611

Appendix C (continued)

	CHE140D	GIL88	MER10	MER17	SEM162	SEM165	SEM163	SEM164	SEM166	DAN78	DAN77	TKA109	TKA110	TKS130	TKS129	TKS131	BAT43	POR96	TKR133	TKS127B	BAT7A	POR32	MER18	MER11
CHE140D	N																							
GIL88	0.03789	N																						
MER10	0.07649	0.06459	N																					
MER17	0.07426	0.06385	0.00297	N																				
SEM162	0.07087	0.06341	0.00894	0.00745	N																			
SEM165	0.07055	0.0631	0.00891	0.00742	0	N																		
SEM163	0.07055	0.0631	0.00891	0.00742	0	0	N																	
SEM164	0.07055	0.0631	0.00891	0.00742	0	0	0	N																
SEM166	0.07055	0.0631	0.00891	0.00742	0	0	0	0	N															
DAN78	0.07419	0.06425	0.01912	0.02065	0.01995	0.01987	0.01987	0.01987	0.01987	N														
DAN77	0.07512	0.06469	0.01858	0.02007	0.0194	0.01933	0.01933	0.01933	0.01933	0	N													
TKA109	0.075	0.06459	0.01856	0.02004	0.01937	0.0193	0.0193	0.0193	0.0193	0	0	N												
TKA110	0.075	0.06459	0.01856	0.02004	0.01937	0.0193	0.0193	0.0193	0.0193	0	0	0	N											
TKS130	0.075	0.06459	0.01856	0.02004	0.01937	0.0193	0.0193	0.0193	0.0193	0	0	0	0	N										
TKS129	0.075	0.06459	0.02004	0.02153	0.02087	0.02079	0.02079	0.02079	0.02079	0.00153	0.00149	0.00148	0.00148	0.00148	N									
TKS131	0.07426	0.06385	0.0193	0.02079	0.02012	0.02004	0.02004	0.02004	0.02004	0.00076	0.00074	0.00074	0.00074	0.00074	0.00074	N								
BAT43	0.07426	0.06385	0.0193	0.02079	0.02012	0.02004	0.02004	0.02004	0.02004	0.00076	0.00074	0.00074	0.00074	0.00074	0.00074	0	N							
POR96	0.07222	0.06327	0.01859	0.02008	0.01941	0.01932	0.01932	0.01932	0.01932	0.00461	0.00448	0.00447	0.00447	0.00447	0.00596	0.00522	0.00522	N						
TKR133	0.07427	0.06162	0.02004	0.02301	0.02237	0.02227	0.02227	0.02227	0.02227	0.01453	0.01413	0.01411	0.01411	0.01411	0.01559	0.01485	0.01485	0.00817	N					
TKS127B	0.07427	0.06162	0.02004	0.02301	0.02237	0.02227	0.02227	0.02227	0.02227	0.01453	0.01413	0.01411	0.01411	0.01411	0.01559	0.01485	0.01485	0.00817	0	N				
BAT7A	0.07427	0.06385	0.02004	0.02153	0.02087	0.02079	0.02079	0.02079	0.02079	0.01223	0.0119	0.01188	0.01188	0.01188	0.01336	0.01262	0.01262	0.00594	0.00668	0.00668	N			
POR32	0.07325	0.06352	0.01716	0.02015	0.01942	0.0194	0.0194	0.0194	0.0194	0.01381	0.01343	0.01342	0.01342	0.01342	0.01492	0.01417	0.01417	0.00748	0.00745	0.00745	0.00671	N		
MER18	0.07278	0.0631	0.01707	0.02004	0.01939	0.0193	0.0193	0.0193	0.0193	0.01376	0.01338	0.01336	0.01336	0.01336	0.01485	0.01411	0.01411	0.00743	0.00742	0.00742	0.00668	0	N	
MER11	0.07324	0.06426	0.0179	0.02089	0.02016	0.02014	0.02014	0.02014	0.02014	0.01383	0.01418	0.01417	0.01417	0.01417	0.01567	0.01492	0.01492	0.00823	0.0082	0.0082	0.00746	0.00075	0.00074	N
MER71	0.07278	0.0631	0.01707	0.02004	0.01939	0.0193	0.0193	0.0193	0.0193	0.01376	0.01338	0.01336	0.01336	0.01336	0.01485	0.01411	0.01411	0.00743	0.00742	0.00742	0.00668	0	0	0.00074
PTL35	0.07278	0.0631	0.01707	0.02004	0.01939	0.0193	0.0193	0.0193	0.0193	0.01376	0.01338	0.01336	0.01336	0.01336	0.01485	0.01411	0.01411	0.00743	0.00742	0.00742	0.00668	0	0	0.00074
MER16	0.07317	0.06337	0.01732	0.02034	0.01962	0.0196	0.0196	0.0196	0.0196	0.0138	0.01357	0.01356	0.01356	0.01356	0.01507	0.01432	0.01432	0.00754	0.00755	0.00755	0.00679	0	0	0.00075
MER13	0.07149	0.06179	0.01561	0.01859	0.01794	0.01785	0.01785	0.01785	0.01785	0.01224	0.0119	0.0119	0.0119	0.0119	0.01339	0.01264	0.01264	0.00596	0.00595	0.00595	0.00594	0	0	0.00074
POR31	0.0713	0.06162	0.01559	0.01856	0.0179	0.01782	0.01782	0.01782	0.01782	0.01223	0.0119	0.01188	0.01188	0.01188	0.01336	0.01262	0.01262	0.00521	0.00742	0.00742	0.00742	0.00449	0.00445	0.00523
CHE140C	0.07093	0.06196	0.01565	0.01864	0.01792	0.01789	0.01789	0.01789	0.01789	0.01228	0.01194	0.01192	0.01192	0.01192	0.01342	0.01267	0.01267	0.00523	0.00745	0.00745	0.00745	0.00449	0.00447	0.00523
POR168	0.0713	0.06162	0.01559	0.01856	0.0179	0.01782	0.01782	0.01782	0.01782	0.01223	0.0119	0.01188	0.01188	0.01188	0.01336	0.01262	0.01262	0.00521	0.00742	0.00742	0.00742	0.00449	0.00445	0.00523
CHE139A	0.0713	0.06162	0.01559	0.01856	0.0179	0.01782	0.01782	0.01782	0.01782	0.01223	0.0119	0.01188	0.01188	0.01188	0.01336	0.01262	0.01262	0.00521	0.00742	0.00742	0.00742	0.00449	0.00445	0.00523
SEM147	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
SEM161A	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
KUN148	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
KUN149	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
SEM151	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
SEM152A	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
SEM156	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
SEM144C	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
SEM158A	0.0713	0.06236	0.01633	0.0193	0.01865	0.01856	0.01856	0.01856	0.01856	0.01299	0.01264	0.01262	0.01262	0.01262	0.01411	0.01336	0.01336	0.00595	0.00817	0.00817	0.00817	0.00524	0.0052	0.00598
SEM160A	0.07056	0.0631	0.01707	0.02004	0.01939	0.0193	0.0193	0.0193	0.0193	0.01223	0.0119	0.01188	0.01188	0.01188	0.01336	0.01262	0.01262	0.0052	0.00891	0.00891	0.00891	0.00598	0.00594	0.00672
MER23	0.07088	0.06183	0.01582	0.01884	0.0182	0.01811	0.01811	0.01811	0.01811	0.01223	0.01207	0.01206	0.01206	0.01206	0.01358	0.01282	0.01282	0.00529	0.00755	0.00755	0.00754	0.00455	0.00452	0.0053
CHE3	0.07064	0.06153	0.01591	0.01895	0.01821	0.01819	0.01819	0.01819	0.01819	0.01227	0.01213	0.01212	0.01212	0.01212	0.01364	0.01288	0.01288	0.00531	0.00757	0.00757	0.00758	0.00456	0.00455	0.00532
MER22	0.07215	0.06245	0.01635	0.01932	0.01867	0.01858	0.01858	0.01858	0.01858	0.01299	0.01264	0.01263	0.01263	0.01263	0.01412	0.01337	0.01337	0.00595	0.00817	0.00817	0.00817	0.00523	0.0052	0.00598
CHE142	0.0713	0.06162	0.01559	0.01856	0.0179	0.01782	0.01782	0.01782	0.01782	0.01223	0.0119	0.01188	0.01188	0.01188	0.01336	0.01262	0.01262	0.00521	0.00742	0.00742	0.00742	0.00449	0.00445	0.00523
CHE138E	0.07779	0.0653	0.03574	0.03886	0.03831	0.03811	0.03811	0.03811	0.03811	0.03265	0.03267	0.03266	0.03266	0.03266	0.03422	0.03343	0.03343	0.02804	0.01943	0.01943	0.02641	0.02734	0.02718	0.02732
Hap.saxi	0.1025	0.09725	0.10393	0.10393	0.10587	0.10542	0.10542	0.10542	0.10542	0.10473	0.10483	0.10468	0.10468	0.10468	0.10616	0.10542	0.10542	0.10196	0.10245	0.10245	0.10319	0.10309	0.10245	0.10383
LAY54	0.12862	0.12185	0.12632	0.12632	0.12759	0.12709	0.12709	0.12709	0.12709	0.1243	0.12407	0.12409	0.12409	0.12409	0.12559	0.12484	0.12484	0.12058	0.12483	0.12483	0.12332	0.12307	0.12256	0.12388
Nem.ting	0.15859	0.1629	0.17478	0.17482	0.17313	0.17313	0.17313	0.17313	0.17313	0.17475	0.17475	0.17474	0.17474	0.17474	0.17474	0.1739	0.1739	0.17091	0.17218	0.17218	0.17558	0.17565	0.1756	0.17563
Heri.fluv	0.16733	0.15524	0.17858	0.17784	0.17728	0.17636	0.17636	0.17636	0.17636	0.17704	0.17787	0.17785	0.17785	0.17785	0.17785	0.1771	0.1771	0.17517	0.17862	0.17862	0.1786	0.17733	0.17634	0.17668

Appendix C (continued)

	CHE140D	GIL88	MER10	MER17	SEM162	SEM165	SEM163	SEM164	SEM166	DAN78	DAN77	TKA109	TKA110	TKS130	TKS129	TKS131	BAT43	POR96	TKR133	TKS127B	BAT7A	POR32	MER18	MER11
Pse.roscf	0.17311	0.16746	0.18711	0.18715	0.18467	0.18468	0.18468	0.18468	0.18468	0.18298	0.18297	0.18296	0.18296	0.18296	0.18462	0.18379	0.18379	0.18155	0.18134	0.18134	0.18134	0.18223	0.18218	0.1822
MER91	0.16784	0.1663	0.17892	0.17743	0.17758	0.17743	0.17743	0.17743	0.17743	0.18041	0.17775	0.17743	0.17743	0.17743	0.17743	0.17669	0.17669	0.17471	0.17372	0.17372	0.17595	0.17648	0.17595	0.17723
MER95	0.16784	0.1663	0.17892	0.17743	0.17758	0.17743	0.17743	0.17743	0.17743	0.18041	0.17775	0.17743	0.17743	0.17743	0.17743	0.17669	0.17669	0.17471	0.17372	0.17372	0.17595	0.17648	0.17595	0.17723
CHE6	0.16784	0.1663	0.17892	0.17743	0.17758	0.17743	0.17743	0.17743	0.17743	0.18041	0.17775	0.17743	0.17743	0.17743	0.17743	0.17669	0.17669	0.17471	0.17372	0.17372	0.17595	0.17648	0.17595	0.17723
CHE7	0.16784	0.1663	0.17892	0.17743	0.17758	0.17743	0.17743	0.17743	0.17743	0.18041	0.17775	0.17743	0.17743	0.17743	0.17743	0.17669	0.17669	0.17471	0.17372	0.17372	0.17595	0.17648	0.17595	0.17723
POR100	0.16784	0.1663	0.17892	0.17743	0.17758	0.17743	0.17743	0.17743	0.17743	0.18041	0.17775	0.17743	0.17743	0.17743	0.17743	0.17669	0.17669	0.17471	0.17372	0.17372	0.17595	0.17648	0.17595	0.17723
MER21	0.16561	0.16407	0.17669	0.1752	0.17615	0.1752	0.1752	0.1752	0.1752	0.17811	0.17552	0.1752	0.1752	0.1752	0.1752	0.17446	0.17446	0.17246	0.17149	0.17149	0.17372	0.17504	0.17372	0.17578
POR27	0.16636	0.16481	0.17743	0.17595	0.17689	0.17595	0.17595	0.17595	0.17595	0.17887	0.17626	0.17595	0.17595	0.17595	0.17595	0.1752	0.1752	0.1732	0.17223	0.17223	0.17446	0.17578	0.17446	0.17653
CHE4	0.16565	0.16411	0.17673	0.17525	0.17619	0.17525	0.17525	0.17525	0.17525	0.17815	0.17556	0.17524	0.17524	0.17524	0.17524	0.1745	0.1745	0.1725	0.17153	0.17153	0.17376	0.17508	0.17376	0.17583
Por.fluv	0.15823	0.14417	0.16092	0.16104	0.1592	0.1581	0.1581	0.1581	0.1581	0.16311	0.16315	0.16311	0.16311	0.16311	0.16309	0.16206	0.16206	0.15828	0.15996	0.15996	0.16099	0.16008	0.15886	0.16012
Petro.mac	0.16046	0.16201	0.1821	0.18134	0.17676	0.17669	0.17669	0.17669	0.17669	0.17362	0.17358	0.17358	0.17358	0.17358	0.17358	0.17281	0.17281	0.17163	0.17358	0.17358	0.17203	0.17293	0.1728	0.17299
Dipsimplx	0.17972	0.1717	0.19389	0.19564	0.19493	0.19476	0.19476	0.19476	0.19476	0.19216	0.19214	0.19212	0.19212	0.19212	0.19208	0.19123	0.19123	0.19159	0.19386	0.19386	0.19293	0.19233	0.19206	0.1924
DipspB	0.20199	0.19302	0.21084	0.21232	0.21264	0.21158	0.21158	0.21158	0.21158	0.19489	0.20591	0.20638	0.20638	0.20638	0.20638	0.20564	0.20564	0.20659	0.20861	0.20861	0.20787	0.20777	0.20713	0.20784
DipspC	0.176	0.16852	0.18708	0.18857	0.18877	0.18782	0.18782	0.18782	0.18782	0.18506	0.18295	0.18337	0.18337	0.18337	0.18337	0.18263	0.18263	0.18282	0.18486	0.18486	0.18411	0.18395	0.18337	0.18394
POR169	0.18853	0.19013	0.20477	0.20249	0.20248	0.20247	0.20247	0.20247	0.20247	0.20389	0.20437	0.20475	0.20475	0.20475	0.20475	0.20398	0.20398	0.20271	0.204	0.204	0.203245	0.20372	0.20404	0.20357
TKS126	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
POR26	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
CHD121	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
CHD123	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
POR29	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
POR24	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
CHD118	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
TKS128	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
POR33	0.18787	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
POR94	0.18712	0.18634	0.20193	0.1997	0.20078	0.1997	0.1997	0.1997	0.1997	0.20101	0.20155	0.20193	0.20193	0.20193	0.20193	0.20119	0.20119	0.19994	0.20119	0.20119	0.20045	0.20194	0.20119	0.20195
MER8	0.18712	0.18634	0.20193	0.1997	0.20078	0.1997	0.1997	0.1997	0.1997	0.20101	0.20155	0.20193	0.20193	0.20193	0.20193	0.20119	0.20119	0.19994	0.20119	0.20119	0.20045	0.20194	0.20119	0.20195
GIL42	0.18786	0.18708	0.20267	0.20045	0.20153	0.20045	0.20045	0.20045	0.20045	0.20177	0.2023	0.20267	0.20267	0.20267	0.20267	0.20193	0.20193	0.20069	0.20193	0.20193	0.20119	0.20269	0.20193	0.20269
SEM155	0.18787	0.1856	0.20119	0.19896	0.2004	0.19896	0.19896	0.19896	0.19896	0.20024	0.20081	0.20119	0.20119	0.20119	0.20119	0.20045	0.20045	0.1992	0.20045	0.20045	0.1997	0.2012	0.20045	0.2012
LAB46	0.18716	0.18637	0.19676	0.19378	0.19253	0.19155	0.19155	0.19155	0.19155	0.19486	0.19452	0.19453	0.19453	0.19453	0.19453	0.19378	0.19378	0.1933	0.19379	0.19379	0.19379	0.19633	0.19528	0.19487
RAM65	0.18716	0.18637	0.19676	0.19378	0.19253	0.19155	0.19155	0.19155	0.19155	0.19486	0.19452	0.19453	0.19453	0.19453	0.19453	0.19378	0.19378	0.1933	0.19379	0.19379	0.19379	0.19633	0.19528	0.19487
LAB80	0.18716	0.18487	0.19527	0.19229	0.19103	0.19006	0.19006	0.19006	0.19006	0.19333	0.19303	0.19303	0.19303	0.19303	0.19303	0.19229	0.19229	0.1918	0.19229	0.19229	0.19229	0.19483	0.19379	0.1942
RAM47A	0.18678	0.18451	0.19494	0.19195	0.1907	0.18973	0.18973	0.18973	0.18973	0.19326	0.19272	0.19272	0.19272	0.19272	0.19272	0.19197	0.19197	0.19147	0.19197	0.19197	0.19197	0.1945	0.19346	0.19386
LAB82	0.1866	0.18432	0.19473	0.19175	0.19049	0.18952	0.18952	0.18952	0.18952	0.19329	0.1925	0.1925	0.1925	0.1925	0.1925	0.19176	0.19176	0.19126	0.19176	0.19176	0.19176	0.19429	0.19325	0.19365
LAB84	0.18622	0.18395	0.19437	0.19138	0.19012	0.18916	0.18916	0.18916	0.18916	0.19326	0.19215	0.19215	0.19215	0.19215	0.19215	0.1914	0.1914	0.1909	0.1914	0.1914	0.1914	0.19393	0.19289	0.19329
LAB75	0.18567	0.18338	0.19377	0.19079	0.18953	0.18856	0.18856	0.18856	0.18856	0.19179	0.19154	0.19154	0.19154	0.19154	0.19154	0.19079	0.19079	0.1903	0.1908	0.1908	0.1908	0.19333	0.19229	0.1927
CHE143C	0.1851	0.19084	0.20372	0.20369	0.20081	0.20088	0.20088	0.20088	0.20088	0.20072	0.20038	0.20086	0.20086	0.20086	0.20264	0.20176	0.20176	0.19841	0.20008	0.20008	0.19981	0.20226	0.20275	0.20207
CHE141	0.18487	0.18486	0.19673	0.19451	0.19472	0.19376	0.19376	0.19376	0.19376	0.19494	0.19481	0.19525	0.19525	0.19525	0.19673	0.19599	0.19599	0.19323	0.19451	0.19451	0.19376	0.19661	0.19599	0.19663
CHE139B	0.18487	0.18486	0.19673	0.19451	0.19472	0.19376	0.19376	0.19376	0.19376	0.19494	0.19481	0.19525	0.19525	0.19525	0.19673	0.19599	0.19599	0.19323	0.19451	0.19451	0.19376	0.19661	0.19599	0.19663
SAY134	0.18487	0.18486	0.19673	0.19451	0.19472	0.19376	0.19376	0.19376	0.19376	0.19494	0.19481	0.19525	0.19525	0.19525	0.19673	0.19599	0.19599	0.19323	0.19451	0.19451	0.19376	0.19661	0.19599	0.19663
SAY135	0.18487	0.18486	0.19673	0.19451	0.19472	0.19376	0.19376	0.19376	0.19376	0.19494	0.19481	0.19525	0.19525	0.19525	0.19673	0.19599	0.19599	0.19323	0.19451	0.19451	0.19376	0.19661	0.19599	0.19663
CHE137	0.18487	0.18486	0.19673	0.19451	0.19472	0.19376	0.19376	0.19376	0.19376	0.19494	0.19481	0.19525	0.19525	0.19525	0.19673	0.19599	0.19599	0.19323	0.19451	0.19451	0.19376	0.19661	0.19599	0.19663
SEM144E	0.16932	0.16333	0.1804	0.1804	0.17983	0.17892	0.17892	0.17892	0.17892	0.1812	0.17997	0.17966	0.17966	0.17966	0.17966	0.17892	0.17892	0.17549	0.17669	0.17669	0.17743	0.17573	0.17446	0.17498
POR99	0.16932	0.16333	0.1804	0.1804	0.17983	0.17892	0.17892	0.17892	0.17892	0.1812	0.17997	0.17966	0.17966	0.17966	0.17966	0.17892	0.17892	0.17549	0.17669	0.17669	0.17743	0.17573	0.17446	0.17498
POR167	0.16932	0.16333	0.1804	0.1804	0.17983	0.17892	0.17892	0.17892	0.17892	0.1812	0.17997	0.17966	0.17966	0.17966	0.17966	0.17892	0.17892	0.17549	0.17669	0.17669	0.17743	0.17573	0.17446	0.17498
MER64	0.16858	0.16258	0.17892	0.17892	0.17834	0.17743	0.17743	0.17743	0.17743	0.17968	0.17848	0.17817	0.17817	0.17817	0.17817	0.17743	0.17743	0.17401	0.1752	0.1752	0.17595	0.17424	0.17298	0.17349
LAY52	0.1708	0.16333	0.17817	0.17966	0.1776	0.17669	0.17669	0.17669	0.17669	0.17891	0.17774	0.17743	0.17743	0.17743	0.17743	0.17669	0.17669	0.17326	0.17446	0.17446	0.1752	0.1735	0.17223	0.17275
GIL67	0.17173	0.16496	0.17926	0.18077	0.17872	0.17778	0.17778	0.17778	0.17778	0.17867	0.17853	0.17852	0.17852	0.17852	0.17852	0.17777	0.17777	0.17428	0.17551	0.17551	0.17625	0.17422	0.17324	0.17361
LAY51	0.1708	0.16333	0.17817	0.17966	0.1776	0.17669	0.17669	0.17669	0.17669	0.17891	0.17774	0.17743	0.17743	0.17743	0.17743	0.17669	0.17669	0.17326	0.17446	0.17446	0.1752	0.1735	0.17223	0.17275
GIL48B	0.1708	0.16333	0.17817	0.17966	0.1776	0.17669	0.17669	0.17669	0.17669	0.17891	0.17774	0.17743	0.17743	0.17743	0.17743	0.17669	0.17669	0.17326	0.17446	0.17446	0.1752	0.1735	0.17223	0.17275
CHE2	0.16709	0.1611	0.17743	0.17743	0.17685	0.17595	0.17595	0.17595	0.17595	0.17815	0.17699	0.17669	0.17669	0.17669	0.17669	0.17595	0.17595	0.17252	0.17372	0.17372	0.17446	0.17274	0.17149	0.172

Appendix C (continued)

	MER71	PTL35	MER16	MER13	POR31	CHE140C	POR168	CHE139A	SEM147	SEM161A	KUN148	KUN149	SEM151	SEM152A	SEM156	SEM144C	SEM158A	SEM160A	MER23	CHE3	MER22	CHE142	CHE138E	Hap.saxi
MER71	N																							
PTL35	0	N																						
MER16	0	0	N																					
MER13	0	0	0	N																				
POR31	0.00445	0.00445	0.00452	0.00298	N																			
CHE140C	0.00447	0.00447	0.00453	0.00299	0	N																		
POR168	0.00445	0.00445	0.00452	0.00298	0	0	N																	
CHE139A	0.00445	0.00445	0.00452	0.00298	0	0	0	N																
SEM147	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	N															
SEM161A	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0	N														
KUN148	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0	0	N													
KUN149	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0	0	0	N												
SEM151	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0	0	0	0	N											
SEM152A	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0	0	0	0	0	N										
SEM156	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0	0	0	0	0	0	N									
SEM144C	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0	0	0	0	0	0	0	N								
SEM158A	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0	0	0	0	0	0	0	0	N							
SEM160A	0.00594	0.00594	0.00603	0.00446	0.00148	0.00149	0.00148	0.00148	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074	N						
MER23	0.00452	0.00452	0.00453	0.00303	0	0	0	0	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00151	N					
CHE3	0.00455	0.00455	0.00454	0.00304	0	0	0	0	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00076	0.00152	0	N				
MER22	0.0052	0.0052	0.00528	0.00372	0.00074	0.00075	0.00074	0.00074	0.00149	0.00149	0.00149	0.00149	0.00149	0.00149	0.00149	0.00149	0.00149	0.00223	0.00076	0.00076	N			
CHE142	0.00445	0.00445	0.00452	0.00298	0	0	0	0	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074	0.00148	0	0	0.00074	N		
CHE138E	0.02718	0.02718	0.02726	0.02567	0.02717	0.02733	0.02717	0.02717	0.02795	0.02795	0.02795	0.02795	0.02795	0.02795	0.02795	0.02795	0.02795	0.02874	0.02717	0.02732	0.02796	0.02717	N	
Hap.saxi	0.10245	0.10245	0.10325	0.1012	0.09948	0.10001	0.09948	0.09948	0.09874	0.09874	0.09874	0.09874	0.09874	0.09874	0.09874	0.09874	0.09874	0.09948	0.09947	0.09942	0.10036	0.09948	0.09939	N
LAY54	0.12256	0.12256	0.12287	0.12112	0.12181	0.1224	0.12181	0.12181	0.12105	0.12105	0.12105	0.12105	0.12105	0.12105	0.12105	0.12105	0.12105	0.1203	0.12192	0.12192	0.12253	0.12181	0.12014	0.13529
Nem.ting	0.1756	0.1756	0.1754	0.17409	0.1722	0.17227	0.1722	0.1722	0.17221	0.17221	0.17221	0.17221	0.17221	0.17221	0.17221	0.17221	0.17221	0.17223	0.17207	0.17225	0.17221	0.1722	0.17068	0.15941
Heri.fluv	0.17634	0.17634	0.17705	0.17578	0.17407	0.17517	0.17407	0.17407	0.17332	0.17332	0.17332	0.17332	0.17332	0.17332	0.17332	0.17332	0.17332	0.17407	0.17395	0.17444	0.17484	0.17407	0.17309	0.15818
Pse.roscf	0.18218	0.18218	0.18196	0.1814	0.18052	0.18058	0.18052	0.18052	0.17971	0.17971	0.17971	0.17971	0.17971	0.17971	0.17971	0.17971	0.17971	0.1797	0.18036	0.18056	0.18054	0.18052	0.17636	0.17235
MER91	0.17595	0.17595	0.17794	0.17492	0.17223	0.17258	0.17223	0.17223	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17223	0.17419	0.174	0.17328	0.17223	0.17431	0.17001
MER95	0.17595	0.17595	0.17794	0.17492	0.17223	0.17258	0.17223	0.17223	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17223	0.17419	0.174	0.17328	0.17223	0.17431	0.17001
CHE6	0.17595	0.17595	0.17794	0.17492	0.17223	0.17258	0.17223	0.17223	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17223	0.17419	0.174	0.17328	0.17223	0.17431	0.17001
CHE7	0.17595	0.17595	0.17794	0.17492	0.17223	0.17258	0.17223	0.17223	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17223	0.17419	0.174	0.17328	0.17223	0.17431	0.17001
POR100	0.17595	0.17595	0.17794	0.17492	0.17223	0.17258	0.17223	0.17223	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17223	0.17419	0.174	0.17328	0.17223	0.17431	0.17001
MER21	0.17372	0.17372	0.17647	0.17269	0.17001	0.17114	0.17001	0.17001	0.16927	0.16927	0.16927	0.16927	0.16927	0.16927	0.16927	0.16927	0.16927	0.17001	0.17192	0.17253	0.17105	0.17001	0.172	0.16778
POR27	0.17446	0.17446	0.17723	0.17344	0.17075	0.17188	0.17075	0.17075	0.17001	0.17001	0.17001	0.17001	0.17001	0.17001	0.17001	0.17001	0.17001	0.17075	0.17267	0.17328	0.17105	0.17075	0.17277	0.16852
CHE4	0.17376	0.17376	0.17651	0.17273	0.17004	0.17118	0.17004	0.17004	0.1693	0.1693	0.1693	0.1693	0.1693	0.1693	0.1693	0.1693	0.1693	0.17004	0.17195	0.17257	0.17109	0.17004	0.17205	0.16782
Por.fluv	0.15886	0.15886	0.15975	0.15701	0.15568	0.157	0.15568	0.15568	0.15565	0.15565	0.15565	0.15565	0.15565	0.15565	0.15565	0.15565	0.15565	0.15673	0.15559	0.15686	0.15674	0.15568	0.15371	0.15934
Petro.mac	0.1728	0.1728	0.17262	0.17209	0.17125	0.1715	0.17125	0.17125	0.17126	0.17126	0.17126	0.17126	0.17126	0.17126	0.17126	0.17126	0.17126	0.17048	0.17102	0.17137	0.17047	0.17125	0.16949	0.16656
Dipsimplx	0.19206	0.19206	0.19196	0.19136	0.19027	0.19068	0.19027	0.19027	0.18937	0.18937	0.18937	0.18937	0.18937	0.18937	0.18937	0.18937	0.18937	0.18941	0.19006	0.19051	0.19112	0.19027	0.1955	0.17865
DipspB	0.20713	0.20713	0.20402	0.20606	0.20564	0.20693	0.20564	0.20564	0.2049	0.2049	0.2049	0.2049	0.2049	0.2049	0.2049	0.2049	0.2049	0.2049	0.19948	0.20028	0.20591	0.20564	0.1945	0.20119
DipspC	0.18337	0.18337	0.18469	0.18234	0.18189	0.18303	0.18189	0.18189	0.18114	0.18114	0.18114	0.18114	0.18114	0.18114	0.18114	0.18114	0.18114	0.18114	0.18317	0.18386	0.18219	0.18189	0.18744	0.17446
POR169	0.20404	0.20404	0.20321	0.2023	0.20174	0.20184	0.20174	0.20174	0.20175	0.20175	0.20175	0.20175	0.20175	0.20175	0.20175	0.20175	0.20175	0.20252	0.20119	0.20082	0.20136	0.20174	0.20216	0.18712
TKS126	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634
POR26	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634
CHD121	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634
CHD123	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634
POR29	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634
POR24	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634
CHD118	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634
TKS128	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634
POR33	0.20193	0.20193	0.20219	0.20023	0.1997	0.20101	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.20005	0.1997	0.20002	0.18634

Appendix C (continued)

	MER71	PTL35	MER16	MER13	POR31	CHE140C	POR168	CHE139A	SEM147	SEM161A	KUN148	KUN149	SEM151	SEM152A	SEM156	SEM144C	SEM158A	SEM160A	MER23	CHE3	MER22	CHE142	CHE138E	Hap. saxi
POR94	0.20119	0.20119	0.20143	0.19948	0.19896	0.20027	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.1997	0.19842	0.19914	0.19931	0.19896	0.19924	0.1856
MER8	0.20119	0.20119	0.20143	0.19948	0.19896	0.20027	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.19896	0.1997	0.19842	0.19914	0.19931	0.19896	0.19924	0.1856
GIL42	0.20193	0.20193	0.20219	0.20022	0.1997	0.20102	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.1997	0.20045	0.19917	0.1999	0.19931	0.1997	0.20001	0.18634
SEM155	0.20045	0.20045	0.20068	0.19874	0.19822	0.19952	0.19822	0.19822	0.19822	0.19822	0.19822	0.19822	0.19822	0.19822	0.19822	0.19822	0.19822	0.19896	0.19766	0.19838	0.19857	0.19822	0.19846	0.18486
LAB46	0.19528	0.19528	0.196	0.19395	0.19305	0.19341	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.1938	0.19371	0.19442	0.19379	0.19305	0.19206	0.1811
RAM65	0.19528	0.19528	0.196	0.19395	0.19305	0.19341	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.19305	0.1938	0.19371	0.19442	0.19379	0.19305	0.19206	0.1811
LAB80	0.19379	0.19379	0.1945	0.19246	0.19155	0.19274	0.19155	0.19155	0.19156	0.19156	0.19156	0.19156	0.19156	0.19156	0.19156	0.19156	0.19156	0.1923	0.19221	0.1929	0.1923	0.19155	0.19051	0.1796
RAM47A	0.19346	0.19346	0.19443	0.19212	0.19121	0.19239	0.19121	0.19121	0.19121	0.19121	0.19121	0.19121	0.19121	0.19121	0.19121	0.19121	0.19121	0.19196	0.19214	0.19283	0.19195	0.19121	0.19043	0.17922
LAB82	0.19325	0.19325	0.19447	0.19192	0.19101	0.19219	0.19101	0.19101	0.19101	0.19101	0.19101	0.19101	0.19101	0.19101	0.19101	0.19101	0.19101	0.19176	0.19218	0.19287	0.19175	0.19101	0.19058	0.17904
LAB84	0.19289	0.19289	0.19442	0.19156	0.19065	0.19182	0.19065	0.19065	0.19065	0.19065	0.19065	0.19065	0.19065	0.19065	0.19065	0.19065	0.19065	0.1914	0.19214	0.19283	0.19139	0.19065	0.19043	0.17864
LAB75	0.19229	0.19229	0.193	0.19097	0.19006	0.19123	0.19006	0.19006	0.19006	0.19006	0.19006	0.19006	0.19006	0.19006	0.19006	0.19006	0.19006	0.19081	0.19071	0.19138	0.1908	0.19006	0.18895	0.17811
CHE143C	0.20275	0.20275	0.20261	0.20066	0.19919	0.1992	0.19919	0.19919	0.1992	0.1992	0.1992	0.1992	0.1992	0.1992	0.1992	0.1992	0.1992	0.19919	0.19931	0.1989	0.19875	0.19919	0.20114	0.185
CHE141	0.19599	0.19599	0.19675	0.1942	0.19376	0.19498	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19451	0.19377	0.19452	0.19406	0.19376	0.19526	0.18263
CHE139B	0.19599	0.19599	0.19675	0.1942	0.19376	0.19498	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19451	0.19377	0.19452	0.19406	0.19376	0.19526	0.18263
SAY134	0.19599	0.19599	0.19675	0.1942	0.19376	0.19498	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19451	0.19377	0.19452	0.19406	0.19376	0.19526	0.18263
SAY135	0.19599	0.19599	0.19675	0.1942	0.19376	0.19498	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19451	0.19377	0.19452	0.19406	0.19376	0.19526	0.18263
CHE137	0.19599	0.19599	0.19675	0.1942	0.19376	0.19498	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19376	0.19451	0.19377	0.19452	0.19406	0.19376	0.19526	0.18263
SEM144E	0.17446	0.17446	0.17643	0.17418	0.17446	0.17559	0.17446	0.17446	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17446	0.17569	0.17632	0.1755	0.17446	0.17427	0.17075
POR99	0.17446	0.17446	0.17643	0.17418	0.17446	0.17559	0.17446	0.17446	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17446	0.17569	0.17632	0.1755	0.17446	0.17427	0.17075
POR167	0.17446	0.17446	0.17643	0.17418	0.17446	0.17559	0.17446	0.17446	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17372	0.17446	0.17569	0.17632	0.1755	0.17446	0.17427	0.17075
MER64	0.17298	0.17298	0.17493	0.17269	0.17298	0.17409	0.17298	0.17298	0.17223	0.17223	0.17223	0.17223	0.17223	0.17223	0.17223	0.17223	0.17223	0.17298	0.17419	0.1748	0.17401	0.17298	0.17271	0.16852
LAY52	0.17223	0.17223	0.17418	0.17195	0.17223	0.17336	0.17223	0.17223	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17223	0.17345	0.17406	0.17327	0.17223	0.17194	0.16927
GIL67	0.17324	0.17324	0.17393	0.17265	0.17324	0.17438	0.17324	0.17324	0.17248	0.17248	0.17248	0.17248	0.17248	0.17248	0.17248	0.17248	0.17248	0.17324	0.1732	0.17381	0.17399	0.17324	0.17171	0.17168
LAY51	0.17223	0.17223	0.17418	0.17195	0.17223	0.17336	0.17223	0.17223	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17223	0.17345	0.17406	0.17327	0.17223	0.17194	0.16927
GIL48B	0.17223	0.17223	0.17418	0.17195	0.17223	0.17336	0.17223	0.17223	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17149	0.17223	0.17345	0.17406	0.17327	0.17223	0.17194	0.16927
CHE2	0.17149	0.17149	0.17342	0.1712	0.17149	0.1726	0.17149	0.17149	0.17075	0.17075	0.17075	0.17075	0.17075	0.17075	0.17075	0.17075	0.17075	0.17149	0.17268	0.17328	0.17253	0.17149	0.17115	0.16704

Appendix C (continued)

	LAY54	Nem.ting	Heri.fluv	Pse.roscf	MER91	MER95	CHE6	CHE7	POR100	MER21	POR27	CHE4	Por.fluv	Petro.mac	Dipsimplx	DipspB	DipspC	POR169	TKS126	POR26	CHD121	CHD123	POR29	POR24
LAY54	N																							
Nem.ting	0.175	N																						
Heri.fluv	0.17232	0.16767	N																					
Pse.roscf	0.18434	0.16636	0.10588	N																				
MER91	0.19536	0.18054	0.13943	0.14657	N																			
MER95	0.19536	0.18054	0.13943	0.14657	0	N																		
CHE6	0.19536	0.18054	0.13943	0.14657	0	0	N																	
CHE7	0.19536	0.18054	0.13943	0.14657	0	0	0	N																
POR100	0.19536	0.18054	0.13943	0.14657	0	0	0	0	N															
MER21	0.19309	0.18068	0.13716	0.14675	0.00223	0.00223	0.00223	0.00223	0.00223	N														
POR27	0.19384	0.18072	0.13791	0.14678	0.00297	0.00297	0.00297	0.00297	0.00297	0.00074	N													
CHE4	0.19313	0.18075	0.13715	0.14679	0.00223	0.00223	0.00223	0.00223	0.00223	0	0.00074	N												
Por.fluv	0.17911	0.17148	0.13334	0.15382	0.12618	0.12618	0.12618	0.12618	0.12618	0.12294	0.12401	0.12302	N											
Petro.mac	0.18329	0.16127	0.15388	0.15068	0.14414	0.14414	0.14414	0.14414	0.14414	0.14569	0.14569	0.14574	0.13996	N										
Dipsimplx	0.19367	0.17879	0.156	0.1606	0.15632	0.15632	0.15632	0.15632	0.15632	0.15447	0.15537	0.15454	0.14959	0.1634	N									
DipspB	0.21	0.19341	0.17327	0.17004	0.17966	0.17966	0.17966	0.17966	0.17966	0.17743	0.17817	0.17749	0.15467	0.16746	0.03355	N								
DipspC	0.18784	0.17555	0.15596	0.15594	0.15367	0.15367	0.15367	0.15367	0.15367	0.15145	0.15219	0.1515	0.15767	0.15574	0.02993	0.05271	N							
POR169	0.19848	0.17053	0.16086	0.17592	0.15908	0.15908	0.15908	0.15908	0.15908	0.15917	0.15924	0.15915	0.17395	0.17674	0.14442	0.16767	0.13209	N						
TKS126	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	N					
POR26	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	0	N				
CHD121	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	0	0	N			
CHD123	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	0	0	0	N		
POR29	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	0	0	0	0	N	
POR24	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	0	0	0	0	0	N
CHD118	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	0	0	0	0	0	0
TKS128	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	0	0	0	0	0	0
POR33	0.19467	0.17067	0.1584	0.176	0.16036	0.16036	0.16036	0.16036	0.16036	0.15813	0.15887	0.15813	0.17058	0.17988	0.14126	0.16333	0.12918	0.00077	0	0	0	0	0	0
POR94	0.19391	0.17065	0.15916	0.17519	0.1611	0.1611	0.1611	0.1611	0.1611	0.15887	0.15961	0.15887	0.1717	0.17911	0.14216	0.16407	0.12843	0.00153	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074
MER8	0.19391	0.17065	0.15916	0.17519	0.1611	0.1611	0.1611	0.1611	0.1611	0.15887	0.15961	0.15887	0.1717	0.17911	0.14216	0.16407	0.12843	0.00153	0.00074	0.00074	0.00074	0.00074	0.00074	0.00074
GIL42	0.19466	0.17074	0.15991	0.17528	0.16184	0.16184	0.16184	0.16184	0.16184	0.15961	0.15961	0.15961	0.17275	0.17911	0.14303	0.16481	0.12918	0.00153	0.00148	0.00148	0.00148	0.00148	0.00148	0.00148
SEM155	0.19316	0.1715	0.1584	0.17436	0.1611	0.1611	0.1611	0.1611	0.1611	0.15887	0.15961	0.15887	0.17166	0.1791	0.14126	0.16333	0.12769	0.0023	0.00148	0.00148	0.00148	0.00148	0.00148	0.00148
LAB46	0.19081	0.17094	0.16128	0.17539	0.16554	0.16554	0.16554	0.16554	0.16554	0.16331	0.16405	0.16337	0.17003	0.17201	0.15043	0.16906	0.13194	0.07993	0.08199	0.08199	0.08199	0.08199	0.08199	0.08199
RAM65	0.19081	0.17094	0.16128	0.17539	0.16554	0.16554	0.16554	0.16554	0.16554	0.16331	0.16405	0.16337	0.17003	0.17201	0.15043	0.16906	0.13194	0.07993	0.08199	0.08199	0.08199	0.08199	0.08199	0.08199
LAB80	0.1893	0.17102	0.15978	0.17546	0.16405	0.16405	0.16405	0.16405	0.16405	0.16181	0.16256	0.16188	0.16794	0.17046	0.14865	0.16757	0.13045	0.07988	0.0805	0.0805	0.0805	0.0805	0.0805	0.0805
RAM47A	0.18924	0.17098	0.15972	0.1754	0.16359	0.16359	0.16359	0.16359	0.16359	0.16134	0.16209	0.1614	0.16787	0.17041	0.14861	0.16636	0.12992	0.07924	0.07989	0.07989	0.07989	0.07989	0.07989	0.07989
LAB82	0.18926	0.171	0.15977	0.17543	0.16345	0.16345	0.16345	0.16345	0.16345	0.16121	0.16195	0.16127	0.16795	0.17045	0.14866	0.16698	0.12981	0.07917	0.07982	0.07982	0.07982	0.07982	0.07982	0.07982
LAB84	0.18924	0.17097	0.15972	0.1754	0.16301	0.16301	0.16301	0.16301	0.16301	0.16076	0.16151	0.16083	0.16787	0.1704	0.1486	0.16649	0.13001	0.07932	0.07996	0.07996	0.07996	0.07996	0.07996	0.07996
LAB75	0.18856	0.17118	0.15828	0.17562	0.16479	0.16479	0.16479	0.16479	0.16479	0.16256	0.1633	0.16262	0.16797	0.16891	0.14776	0.16683	0.12971	0.07988	0.07976	0.07976	0.07976	0.07976	0.07976	0.07976
CHE143C	0.19929	0.18565	0.17918	0.17511	0.17289	0.17289	0.17289	0.17289	0.17289	0.1731	0.17315	0.17318	0.18866	0.16893	0.17283	0.18757	0.14095	0.09668	0.09668	0.09668	0.09668	0.09668	0.09668	0.09668
CHE141	0.19389	0.18048	0.17785	0.1741	0.16778	0.16778	0.16778	0.16778	0.16778	0.16852	0.16927	0.16857	0.17296	0.17206	0.16575	0.17817	0.13957	0.0989	0.10022	0.10022	0.10022	0.10022	0.10022	0.10022
CHE139B	0.19389	0.18048	0.17785	0.1741	0.16778	0.16778	0.16778	0.16778	0.16778	0.16852	0.16927	0.16857	0.17296	0.17206	0.16575	0.17817	0.13957	0.0989	0.10022	0.10022	0.10022	0.10022	0.10022	0.10022
SAY134	0.19389	0.18048	0.17785	0.1741	0.16778	0.16778	0.16778	0.16778	0.16778	0.16852	0.16927	0.16857	0.17296	0.17206	0.16575	0.17817	0.13957	0.0989	0.10022	0.10022	0.10022	0.10022	0.10022	0.10022
SAY135	0.19389	0.18048	0.17785	0.1741	0.16778	0.16778	0.16778	0.16778	0.16778	0.16852	0.16927	0.16857	0.17296	0.17206	0.16575	0.17817	0.13957	0.0989	0.10022	0.10022	0.10022	0.10022	0.10022	0.10022
CHE137	0.19389	0.18048	0.17785	0.1741	0.16778	0.16778	0.16778	0.16778	0.16778	0.16852	0.16927	0.16857	0.17296	0.17206	0.16575	0.17817	0.13957	0.0989	0.10022	0.10022	0.10022	0.10022	0.10022	0.10022
SEM144E	0.17661	0.17208	0.1545	0.16752	0.14625	0.14625	0.14625	0.14625	0.14625	0.14402	0.14477	0.14408	0.13967	0.15419	0.16097	0.18411	0.15145	0.15529	0.15516	0.15516	0.15516	0.15516	0.15516	0.15516
POR99	0.17661	0.17208	0.1545	0.16752	0.14625	0.14625	0.14625	0.14625	0.14625	0.14402	0.14477	0.14408	0.13967	0.15419	0.16097	0.18411	0.15145	0.15529	0.15516	0.15516	0.15516	0.15516	0.15516	0.15516
POR167	0.17661	0.17208	0.1545	0.16752	0.14625	0.14625	0.14625	0.14625	0.14625	0.14402	0.14477	0.14408	0.13967	0.15419	0.16097	0.18411	0.15145	0.15529	0.15516	0.15516	0.15516	0.15516	0.15516	0.15516
MER64	0.17436	0.17038	0.15225	0.16504	0.14477	0.14477	0.14477	0.14477	0.14477	0.14254	0.14328	0.14259	0.13966	0.15186	0.1575	0.18114	0.14848	0.15374	0.15367	0.15367	0.15367	0.15367	0.15367	0.15367
LAY52	0.17512	0.1704	0.15452	0.16588	0.14774	0.14774	0.14774	0.14774	0.14774	0.14551	0.14625	0.14556	0.13859	0.15419	0.15747	0.18114	0.14848	0.15374	0.15367	0.15367	0.15367	0.15367	0.15367	0.15367
GIL67	0.17522	0.17021	0.15437	0.16566	0.14837	0.14837	0.14837	0.14837	0.14837	0.1461	0.14686	0.14616	0.13847	0.15399	0.15728	0.17615	0.14758	0.15304	0.15301	0.15301	0.15301	0.15301	0.15301	0.15301
LAY51	0.17512	0.1704	0.15452	0.16588	0.14774	0.14774	0.14774	0.14774	0.14774	0.14551	0.14625	0.14556	0.13859	0.15419	0.15747	0.18114	0.14848	0.15374	0.15367	0.15367	0.15367	0.15367	0.15367	0.15367
GIL48B	0.17512	0.1704	0.15452	0.16588	0.14774	0.14774	0.14774	0.14774	0.14774	0.14551	0.14625	0.14556	0.13859	0.15419	0.15747	0.18114	0.14848	0.15374	0.15367	0.15367	0.15367	0.15367	0.15367	0.15367
CHE2	0.17285	0.17037	0.15073	0.16502	0.14328	0.14328	0.14328	0.14328	0.14328	0.14105	0.1418	0.14111	0.13749	0.15031	0.15573	0.17966	0.14699	0.15369	0.15219	0.15219	0.15219	0.15219	0.15219	0.15219

Appendix C (continued)

	CHD118	TKS128	POR33	POR94	MER8	GIL42	SEM155	LAB46	RAM65	LAB80	RAM47A	LAB82	LAB84	LAB75	CHE143C	CHE141	CHE139B	SAY134	SAY135	CHE137	SEM144E	POR99	POR167	MER64
CHD118	N																							
TKS128	0	N																						
POR33	0	0	N																					
POR94	0.00074	0.00074	0.00074	N																				
MER8	0.00074	0.00074	0.00074	0	N																			
GIL42	0.00148	0.00148	0.00148	0.00074	0.00074	N																		
SEM155	0.00148	0.00148	0.00148	0.00074	0.00074	0.00148	N																	
LAB46	0.08199	0.08199	0.08199	0.08125	0.08125	0.08199	0.08199	N																
RAM65	0.08199	0.08199	0.08199	0.08125	0.08125	0.08199	0.08199	0	N															
LAB80	0.0805	0.0805	0.0805	0.07975	0.07975	0.0805	0.0805	0.00149	0.00149	N														
RAM47A	0.07989	0.07989	0.07989	0.07914	0.07914	0.07989	0.07989	0.00149	0.00149	0	N													
LAB82	0.07982	0.07982	0.07982	0.07907	0.07907	0.07982	0.07982	0.00149	0.00149	0	0	N												
LAB84	0.07996	0.07996	0.07996	0.07922	0.07922	0.07996	0.07996	0.00149	0.00149	0	0	0	N											
LAB75	0.07976	0.07976	0.07976	0.07901	0.07901	0.07976	0.07976	0.00298	0.00298	0.00149	0.00149	0.00149	0.00149	N										
CHE143C	0.09668	0.09668	0.09668	0.0958	0.0958	0.09587	0.09668	0.10929	0.10929	0.10933	0.10878	0.10861	0.10803	0.10945	N									
CHE141	0.10022	0.10022	0.10022	0.09948	0.09948	0.10022	0.10022	0.1147	0.1147	0.1147	0.11424	0.1141	0.11363	0.11396	0.00269	N								
CHE139B	0.10022	0.10022	0.10022	0.09948	0.09948	0.10022	0.10022	0.1147	0.1147	0.1147	0.11424	0.1141	0.11363	0.11396	0.00269	0	N							
SAY134	0.10022	0.10022	0.10022	0.09948	0.09948	0.10022	0.10022	0.1147	0.1147	0.1147	0.11424	0.1141	0.11363	0.11396	0.00269	0	0	N						
SAY135	0.10022	0.10022	0.10022	0.09948	0.09948	0.10022	0.10022	0.1147	0.1147	0.1147	0.11424	0.1141	0.11363	0.11396	0.00269	0	0	0	N					
CHE137	0.10022	0.10022	0.10022	0.09948	0.09948	0.10022	0.10022	0.1147	0.1147	0.1147	0.11424	0.1141	0.11363	0.11396	0.00269	0	0	0	0	N				
SEM144E	0.15516	0.15516	0.15516	0.1559	0.1559	0.15664	0.1559	0.15059	0.15059	0.15059	0.15013	0.14999	0.14952	0.14984	0.15816	0.15516	0.15516	0.15516	0.15516	0.15516	N			
POR99	0.15516	0.15516	0.15516	0.1559	0.1559	0.15664	0.1559	0.15059	0.15059	0.15059	0.15013	0.14999	0.14952	0.14984	0.15816	0.15516	0.15516	0.15516	0.15516	0.15516	0	N		
POR167	0.15516	0.15516	0.15516	0.1559	0.1559	0.15664	0.1559	0.15059	0.15059	0.15059	0.15013	0.14999	0.14952	0.14984	0.15816	0.15516	0.15516	0.15516	0.15516	0.15516	0	0	N	
MER64	0.15367	0.15367	0.15367	0.15442	0.15442	0.15516	0.15442	0.14985	0.14985	0.14984	0.14939	0.14924	0.14878	0.1491	0.15636	0.15367	0.15367	0.15367	0.15367	0.15367	0.00297	0.00297	0.00297	N
LAY52	0.15367	0.15367	0.15367	0.15442	0.15442	0.15516	0.15442	0.14985	0.14985	0.14985	0.14939	0.14925	0.14879	0.1491	0.1545	0.15367	0.15367	0.15367	0.15367	0.15367	0.0052	0.0052	0.0052	0.00371
GIL67	0.15301	0.15301	0.15301	0.15377	0.15377	0.15452	0.15376	0.14834	0.14834	0.14834	0.14825	0.14832	0.14827	0.14759	0.15451	0.15367	0.15367	0.15367	0.15367	0.15367	0.00526	0.00526	0.00526	0.00376
LAY51	0.15367	0.15367	0.15367	0.15442	0.15442	0.15516	0.15442	0.14985	0.14985	0.14985	0.14939	0.14925	0.14879	0.1491	0.1545	0.15367	0.15367	0.15367	0.15367	0.15367	0.0052	0.0052	0.0052	0.00371
GIL48B	0.15367	0.15367	0.15367	0.15442	0.15442	0.15516	0.15442	0.14985	0.14985	0.14985	0.14939	0.14925	0.14879	0.1491	0.1545	0.15367	0.15367	0.15367	0.15367	0.15367	0.0052	0.0052	0.0052	0.00371
CHE2	0.15219	0.15219	0.15219	0.15293	0.15293	0.15367	0.15293	0.14985	0.14985	0.14835	0.14789	0.14775	0.14728	0.14761	0.15636	0.15442	0.15442	0.15442	0.15442	0.15442	0.00445	0.00445	0.00445	0.00148

	LAY52	GIL67	LAY51	GIL48B	CHE2
LAY52	N				
GIL67	0	N			
LAY51	0	0	N		
GIL48B	0	0	0	N	
CHE2	0.0052	0.00527	0.0052	0.0052	N

Appendix D: Uncorrected pairwise distance matrix of the *cox1*-5' gene sequences

	CHE139A	CHE140C	CHE142	CHE3	CHE168	POR31	SEM144C	SEM160A	KUN148	SEM147	SEM149	SEM151	SEM158A	SEM156	SEM161A	SEM152A	CHE138E	TKS127B	TKR133	BAT7A	MER71	POR30	PTL35	MER16
CHE139A	N																							
CHE140C	0.00149	N																						
CHE142	0.00149	0	N																					
CHE3	0.00149	0	0	N																				
CHE168	0.00149	0	0	0	N																			
POR31	0.00149	0	0	0	0	N																		
SEM144C	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	N																	
SEM160A	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	0	N																
KUN148	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	0	0	N															
SEM147	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	0	0	0	N														
SEM149	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	0	0	0	0	N													
SEM151	0.00447	0.00298	0.00298	0.00298	0.00298	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	N												
SEM158A	0.00447	0.00298	0.00298	0.00298	0.00298	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	0	N											
SEM156	0.00447	0.00298	0.00298	0.00298	0.00298	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	0	0	N										
SEM161A	0.00447	0.00298	0.00298	0.00298	0.00298	0.00298	0.00149	0.00149	0.00149	0.00149	0.00149	0	0	0	N									
SEM152A	0.00596	0.00447	0.00447	0.00447	0.00447	0.00447	0.00298	0.00298	0.00298	0.00298	0.00298	0.00149	0.00149	0.00149	0.00149	N								
CHE138E	0.08197	0.08048	0.08048	0.08048	0.08048	0.08048	0.07899	0.07899	0.07899	0.07899	0.07899	0.0775	0.0775	0.0775	0.0775	0.07899	N							
TKS127B	0.08197	0.08048	0.08048	0.08048	0.08048	0.08048	0.07899	0.07899	0.07899	0.07899	0.07899	0.0775	0.0775	0.0775	0.0775	0.07899	0	N						
TKR133	0.08197	0.08048	0.08048	0.08048	0.08048	0.08048	0.07899	0.07899	0.07899	0.07899	0.07899	0.0775	0.0775	0.0775	0.0775	0.07899	0	0	N					
BAT7A	0.07899	0.0775	0.0775	0.0775	0.0775	0.0775	0.07601	0.07601	0.07601	0.07601	0.07601	0.07452	0.7452	0.7452	0.7452	0.07601	0.02832	0.02832	0.02832	N				
MER71	0.09389	0.0924	0.0924	0.0924	0.0924	0.0924	0.0901	0.0901	0.0901	0.0901	0.0901	0.0924	0.0924	0.0924	0.0924	0.09389	0.06557	0.06557	0.06557	0.06557	N			
POR30	0.09389	0.0924	0.0924	0.0924	0.0924	0.0924	0.0901	0.0901	0.0901	0.0901	0.0901	0.0924	0.0924	0.0924	0.0924	0.09389	0.06557	0.06557	0.06557	0.06557	0	N		
PTL35	0.09389	0.0924	0.0924	0.0924	0.0924	0.0924	0.0901	0.0901	0.0901	0.0901	0.0901	0.0924	0.0924	0.0924	0.0924	0.09389	0.06557	0.06557	0.06557	0.06557	0	0	N	
MER16	0.09389	0.0924	0.0924	0.0924	0.0924	0.0924	0.0901	0.0901	0.0901	0.0901	0.0901	0.0924	0.0924	0.0924	0.0924	0.09389	0.06557	0.06557	0.06557	0.06557	0	0	0	N
POR32	0.09389	0.0924	0.0924	0.0924	0.0924	0.0924	0.0901	0.0901	0.0901	0.0901	0.0901	0.0924	0.0924	0.0924	0.0924	0.09389	0.06557	0.06557	0.06557	0.06557	0	0	0	0
MER18	0.0924	0.09091	0.09091	0.09091	0.09091	0.09091	0.08942	0.08942	0.08942	0.08942	0.08942	0.0901	0.09091	0.09091	0.09091	0.0924	0.06408	0.06408	0.06408	0.06408	0.00149	0.00149	0.00149	0.00149
MER13	0.0924	0.09091	0.09091	0.09091	0.09091	0.09091	0.08942	0.08942	0.08942	0.08942	0.08942	0.0901	0.09091	0.09091	0.09091	0.0924	0.06408	0.06408	0.06408	0.06408	0.00149	0.00149	0.00149	0.00149
MER22	0.0924	0.09091	0.09091	0.09091	0.09091	0.09091	0.08942	0.08942	0.08942	0.08942	0.08942	0.0901	0.09091	0.09091	0.09091	0.0924	0.06408	0.06408	0.06408	0.06408	0.00149	0.00149	0.00149	0.00149
MER23	0.0924	0.09091	0.09091	0.09091	0.09091	0.09091	0.08942	0.08942	0.08942	0.08942	0.08942	0.0901	0.09091	0.09091	0.09091	0.0924	0.06408	0.06408	0.06408	0.06408	0.00149	0.00149	0.00149	0.00149
MER11	0.0914	0.08987	0.08987	0.08987	0.08987	0.08987	0.08835	0.08835	0.08835	0.08835	0.08835	0.08988	0.08988	0.08988	0.08988	0.0914	0.06405	0.06405	0.06405	0.06554	0.00154	0.00154	0.00154	0.00154
DAN78	0.10879	0.1073	0.1073	0.1073	0.1073	0.1073	0.10581	0.10581	0.10581	0.10581	0.10581	0.10432	0.10432	0.10432	0.10432	0.10283	0.09538	0.09538	0.09538	0.08495	0.12072	0.12072	0.12072	0.12072
DAN77	0.10879	0.1073	0.1073	0.1073	0.1073	0.1073	0.10581	0.10581	0.10581	0.10581	0.10581	0.10432	0.10432	0.10432	0.10432	0.10283	0.09538	0.09538	0.09538	0.08495	0.12072	0.12072	0.12072	0.12072
TKA109	0.11028	0.10879	0.10879	0.10879	0.10879	0.10879	0.1073	0.1073	0.1073	0.1073	0.1073	0.10581	0.10581	0.10581	0.10581	0.10432	0.09687	0.09687	0.09687	0.08644	0.12221	0.12221	0.12221	0.12221
TKS130	0.11028	0.10879	0.10879	0.10879	0.10879	0.10879	0.1073	0.1073	0.1073	0.1073	0.1073	0.10581	0.10581	0.10581	0.10581	0.10432	0.09687	0.09687	0.09687	0.08644	0.12221	0.12221	0.12221	0.12221
TKA110	0.11028	0.10879	0.10879	0.10879	0.10879	0.10879	0.1073	0.1073	0.1073	0.1073	0.1073	0.10581	0.10581	0.10581	0.10581	0.10432	0.09687	0.09687	0.09687	0.08644	0.12221	0.12221	0.12221	0.12221
MER97	0.11923	0.11773	0.11773	0.11773	0.11773	0.11773	0.11624	0.11624	0.11624	0.11624	0.11624	0.11475	0.11475	0.11475	0.11475	0.11326	0.09687	0.09687	0.09687	0.08942	0.12519	0.12519	0.12519	0.12519
POR96	0.11923	0.11773	0.11773	0.11773	0.11773	0.11773	0.11624	0.11624	0.11624	0.11624	0.11624	0.11475	0.11475	0.11475	0.11475	0.11326	0.09687	0.09687	0.09687	0.08942	0.12519	0.12519	0.12519	0.12519
TKS129	0.11923	0.11773	0.11773	0.11773	0.11773	0.11773	0.11624	0.11624	0.11624	0.11624	0.11624	0.11475	0.11475	0.11475	0.11475	0.11326	0.09687	0.09687	0.09687	0.08942	0.12519	0.12519	0.12519	0.12519
TKS131	0.12072	0.11923	0.11923	0.11923	0.11923	0.11923	0.11773	0.11773	0.11773	0.11773	0.11773	0.11624	0.11624	0.11624	0.11624	0.11475	0.09836	0.09836	0.09836	0.0901	0.1237	0.1237	0.1237	0.1237
BAT43	0.11624	0.11475	0.11475	0.11475	0.11475	0.11475	0.11326	0.11326	0.11326	0.11326	0.11326	0.11177	0.11177	0.11177	0.11177	0.11028	0.09687	0.09687	0.09687	0.08942	0.12221	0.12221	0.12221	0.12221
MER10	0.13413	0.13264	0.13264	0.13264	0.13264	0.13264	0.13115	0.13115	0.13115	0.13115	0.13115	0.12966	0.12966	0.12966	0.12966	0.13115	0.13264	0.13264	0.13264	0.13264	0.1535	0.1535	0.1535	0.1535
MER17	0.13413	0.13264	0.13264	0.13264	0.13264	0.13264	0.13115	0.13115	0.13115	0.13115	0.13115	0.12966	0.12966	0.12966	0.12966	0.13115	0.13264	0.13264	0.13264	0.13264	0.1535	0.1535	0.1535	0.1535
SEM164	0.12519	0.1237	0.1237	0.1237	0.1237	0.1237	0.12221	0.12221	0.12221	0.12221	0.12221	0.12072	0.12072	0.12072	0.12072	0.12221	0.12817	0.12817	0.12817	0.12072	0.14605	0.14605	0.14605	0.14605
SEM166	0.12519	0.1237	0.1237	0.1237	0.1237	0.1237	0.12221	0.12221	0.12221	0.12221	0.12221	0.12072	0.12072	0.12072	0.12072	0.12221	0.12817	0.12817	0.12817	0.12072	0.14605	0.14605	0.14605	0.14605
SEM165	0.12519	0.1237	0.1237	0.1237	0.1237	0.1237	0.12221	0.12221	0.12221	0.12221	0.12221	0.12072	0.12072	0.12072	0.12072	0.12221	0.12817	0.12817	0.12817	0.12072	0.14605	0.14605	0.14605	0.14605
SEM162	0.12519	0.1237	0.1237	0.1237	0.1237	0.1237	0.12221	0.12221	0.12221	0.12221	0.12221	0.12072	0.12072	0.12072	0.12072	0.12221	0.12817	0.12817	0.12817	0.12072	0.14605	0.14605	0.14605	0.14605
SEM163	0.12221	0.12072	0.12072	0.12072	0.12072	0.12072	0.11923	0.11923	0.11923	0.11923	0.11923	0.11773	0.11773	0.11773	0.11773	0.11923	0.12519	0.12519	0.12519	0.11773	0.14307	0.14307	0.14307	0.14307
CHD117A	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.21461	0.21311	0.21311	0.21311	0.21311	0.20715	0.20715	0.20715	0.20715
CHD122A	0.22304	0.22152	0.22152	0.22152	0.22152	0.22152	0.22	0.22	0.22	0.22	0.22	0.22	0.21848	0.21848	0.21848	0.21848	0.21695	0.21531	0.21531	0.21531	0.21535	0.20764	0.20764	0.20764
CHD120	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.21461	0.21311	0.21311	0.21311	0.21311	0.20715	0.20715	0.20715	0.20715
KU1065	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.2161	0.21461	0.21461	0.21461	0.21461	0.21311	0.21162	0.21162	0.21162	0.21162	0.20566	0.20566	0.20566	0.20566
BIS62	0.20864	0.20715	0.20715	0.20715	0.20715	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20268	0.19523	0.19523	0.19523	0.20268	0.20268	0.20268	0.20268	0.20268
BIS63	0.20864	0.20715	0.20715	0.20715	0.20715	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20268	0.19523	0.19523	0.19523	0.20268	0.20268	0.20268	0.20268	0.20268
SEM159A	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20268	0.20268	0.20268	0.20268	0.20119	0.19374	0.19374	0.19374	0.20119	0.19821	0.19821	0.19821	0.19821
SEM161B	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20268	0.20268	0.20268	0.20268	0.20119	0.19374	0.19374	0.19374	0.20119	0.19821	0.19821	0.19821	0.19821
MER14	0.22206	0.22057	0.22057	0.22057	0.22057	0.22057	0.22206	0.22206	0.22206	0.22206	0.22206	0.22057	0.22057	0.22057	0.22057	0.22206	0.22206	0.22206	0.22206	0.22206	0.22057	0.22057	0.22057	0.22057
MER15	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.22057	0.22355	0.22355	0.22355	0.22355	0.21908	0.21908	0.21908	0.21908
CHE1	0.21311	0.21162	0.21162	0.21162	0.21162	0.21162	0.21013	0.21013	0.21013	0.21013	0.21013	0.20864	0.20864	0.20864	0.20864	0.21013	0.20175	0.20175	0.20175	0.21013	0.21311	0.21311	0.21311	0.21311
MesspC	0.19672	0.19523	0.19523	0.19523	0.19523	0.19523	0.19374	0.19374	0.19374	0.19374	0.19374	0.19225	0.19225	0.19225	0.19225	0.19374	0.19672	0.19672	0.19672	0.1997	0.20566	0.20566	0.20566	0.20566
CHE138C	0.19672	0.19523	0.19523	0.19523	0.19523	0.19523	0.19374	0.19374	0.19374	0.19374	0.19374	0.19225	0.19225	0.19225	0.19225	0.19374	0.19672	0.19672	0.19672	0.1997	0.20566	0.20566	0.20566	0.20566
TKS127A	0.19672	0.19523	0.19523	0.19523	0.19523	0.19523	0.19374	0.19374	0.19374	0.19374	0.19374	0.19225	0.19225	0.19225	0.19225	0.19374	0.19672	0.19672	0.19672	0.1997	0.20566	0.20566	0.20566	0.20566

Appendix D (continued)

	CHE139A	CHE140C	CHE142	CHE3	CHE168	POR31	SEM144C	SEM160A	KUN148	SEM147	SEM149	SEM151	SEM158A	SEM156	SEM161A	SEM152A	CHE138E	TKS127B	TKR133	BAT7A	MER71	POR30	PTL35	MER16
CHE143A	0.19672	0.19523	0.19523	0.19523	0.19523	0.19523	0.19374	0.19374	0.19374	0.19374	0.19374	0.19225	0.19225	0.19225	0.19225	0.19374	0.19672	0.19672	0.19672	0.1997	0.20566	0.20566	0.20566	0.20566
TKR132	0.19821	0.19672	0.19672	0.19672	0.19672	0.19672	0.19523	0.19523	0.19523	0.19523	0.19523	0.19374	0.19374	0.19374	0.19374	0.19523	0.19821	0.19821	0.19821	0.20119	0.20715	0.20715	0.20715	0.20715
POR34	0.19523	0.19374	0.19374	0.19374	0.19374	0.19374	0.19225	0.19225	0.19225	0.19225	0.19225	0.19076	0.19076	0.19076	0.19076	0.19225	0.19523	0.19523	0.19523	0.19821	0.20417	0.20417	0.20417	0.20417
KEM112	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20715	0.20715	0.20715	0.20715	0.20566	0.21908	0.21908	0.21908	0.21311	0.22653	0.22653	0.22653	0.22653
CHD122B	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20715	0.20715	0.20715	0.20715	0.20566	0.21908	0.21908	0.21908	0.21311	0.22653	0.22653	0.22653	0.22653
CHD117B	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20715	0.20715	0.20715	0.20715	0.20566	0.21908	0.21908	0.21908	0.21311	0.22653	0.22653	0.22653	0.22653
KEM115	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20715	0.20715	0.20715	0.20715	0.20566	0.21908	0.21908	0.21908	0.21311	0.22653	0.22653	0.22653	0.22653
KEM111	0.20715	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20566	0.20715	0.20715	0.20715	0.20715	0.20566	0.21908	0.21908	0.21908	0.21311	0.22653	0.22653	0.22653	0.22653
KEM113	0.20878	0.20729	0.20729	0.20729	0.20729	0.20729	0.20729	0.20729	0.20729	0.20729	0.20729	0.20878	0.20878	0.20878	0.20878	0.20729	0.21771	0.21771	0.21771	0.21175	0.22517	0.22517	0.22517	0.22517
LEN41	0.21013	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.21013	0.21013	0.21013	0.21013	0.20864	0.21908	0.21908	0.21908	0.21311	0.21759	0.21759	0.21759	0.21759
CHE140D	0.21013	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20715	0.20715	0.20715	0.20715	0.20566	0.2161	0.2161	0.2161	0.21162	0.22653	0.22653	0.22653	0.22653
GIL88	0.20268	0.20119	0.20119	0.20119	0.20119	0.20119	0.20119	0.20119	0.20119	0.20119	0.20119	0.1997	0.1997	0.1997	0.1997	0.19821	0.21162	0.21162	0.21162	0.20864	0.2161	0.2161	0.2161	0.2161
GIL57B	0.20417	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20119	0.20119	0.20119	0.20119	0.1997	0.21311	0.21311	0.21311	0.21013	0.21461	0.21461	0.21461	0.21461
CHE140F	0.18778	0.18629	0.18629	0.18629	0.18629	0.18629	0.18778	0.18778	0.18778	0.18778	0.18778	0.18629	0.18629	0.18629	0.18629	0.18778	0.19523	0.19523	0.19523	0.19672	0.19225	0.19225	0.19225	0.19225
CHE5	0.18927	0.18778	0.18778	0.18778	0.18778	0.18778	0.18927	0.18927	0.18927	0.18927	0.18927	0.18778	0.18778	0.18778	0.18778	0.18927	0.19672	0.19672	0.19672	0.19821	0.19374	0.19374	0.19374	0.19374
LAY86	0.18778	0.18629	0.18629	0.18629	0.18629	0.18629	0.18778	0.18778	0.18778	0.18778	0.18778	0.18629	0.18629	0.18629	0.18629	0.18778	0.19523	0.19523	0.19523	0.19672	0.19225	0.19225	0.19225	0.19225
MER19	0.18949	0.188	0.188	0.188	0.188	0.188	0.18949	0.18949	0.18949	0.18949	0.18949	0.18799	0.18799	0.18799	0.18799	0.18949	0.18955	0.18955	0.18955	0.19103	0.18657	0.18657	0.18657	0.18657
MesspD	0.18331	0.18182	0.18182	0.18182	0.18182	0.18182	0.18331	0.18331	0.18331	0.18331	0.18331	0.18182	0.18182	0.18182	0.18182	0.18331	0.19076	0.19076	0.19076	0.19523	0.19374	0.19374	0.19374	0.19374
GIL38	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20268	0.20268	0.20268	0.20268	0.20268	0.20119	0.20119	0.20119	0.20119	0.20268	0.20566	0.20566	0.20566	0.20417	0.21461	0.21461	0.21461	0.21461
BAT39	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20268	0.20268	0.20268	0.20268	0.20268	0.20119	0.20119	0.20119	0.20119	0.20268	0.20566	0.20566	0.20566	0.20417	0.21461	0.21461	0.21461	0.21461
GIL56	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20268	0.20268	0.20268	0.20268	0.20268	0.20119	0.20119	0.20119	0.20119	0.20268	0.20566	0.20566	0.20566	0.20417	0.21461	0.21461	0.21461	0.21461
FUS3	0.20268	0.20119	0.20119	0.20119	0.20119	0.20119	0.1997	0.1997	0.1997	0.1997	0.1997	0.19821	0.19821	0.19821	0.19821	0.1997	0.20566	0.20566	0.20566	0.20417	0.21461	0.21461	0.21461	0.21461
FUS2	0.20268	0.20119	0.20119	0.20119	0.20119	0.20119	0.1997	0.1997	0.1997	0.1997	0.1997	0.19821	0.19821	0.19821	0.19821	0.1997	0.20566	0.20566	0.20566	0.20417	0.21461	0.21461	0.21461	0.21461
NIP66	0.20417	0.20268	0.20268	0.20268	0.20268	0.20268	0.20119	0.20119	0.20119	0.20119	0.20119	0.1997	0.1997	0.1997	0.1997	0.20119	0.20715	0.20715	0.20715	0.20566	0.2161	0.2161	0.2161	0.2161
SEM159D	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20268	0.20268	0.20268	0.20268	0.20268	0.20119	0.20119	0.20119	0.20119	0.20268	0.20864	0.20864	0.20864	0.20715	0.21759	0.21759	0.21759	0.21759
LAB76	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.21461	0.22653	0.22653	0.22653	0.22653
LAB83	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.21461	0.22653	0.22653	0.22653	0.22653
LAB81	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.21461	0.22653	0.22653	0.22653	0.22653
LAB74	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.21461	0.22653	0.22653	0.22653	0.22653
LAB79	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.21461	0.22653	0.22653	0.22653	0.22653
LAY55	0.20566	0.20417	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20566	0.20566	0.20566	0.20566	0.21461	0.22653	0.22653	0.22653	0.22653
R.fungi	0.24481	0.24328	0.24328	0.24328	0.24328	0.24328	0.24331	0.24331	0.24331	0.24331	0.24331	0.2418	0.2418	0.2418	0.2418	0.24027	0.24468	0.24468	0.24468	0.24014	0.25499	0.25499	0.25499	0.25499
A. japon	0.26578	0.26426	0.26426	0.26426	0.26426	0.26426	0.26428	0.26428	0.26428	0.26428	0.26428	0.26579	0.26579	0.26579	0.26579	0.26428	0.27172	0.27172	0.27172	0.27928	0.27005	0.27005	0.27005	0.27005
CHE4	0.24489	0.24335	0.24335	0.24335	0.24335	0.24335	0.24336	0.24336	0.24336	0.24336	0.24336	0.24182	0.24182	0.24182	0.24182	0.24192	0.25281	0.25281	0.25281	0.25104	0.26641	0.26641	0.26641	0.26641
MER21	0.2459	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	0.24292	0.24292	0.24441	0.25186	0.25186	0.25186	0.24888	0.26379	0.26379	0.26379	0.26379
MER91	0.2459	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	0.24292	0.24292	0.24441	0.25186	0.25186	0.25186	0.24888	0.26379	0.26379	0.26379	0.26379
CHE6	0.2459	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	0.24292	0.24292	0.24441	0.25186	0.25186	0.25186	0.24888	0.26379	0.26379	0.26379	0.26379
POR27	0.2459	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	0.24292	0.24292	0.24441	0.25186	0.25186	0.25186	0.24888	0.26379	0.26379	0.26379	0.26379
POR100	0.2459	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	0.24292	0.24292	0.24441	0.25186	0.25186	0.25186	0.24888	0.26379	0.26379	0.26379	0.26379
MER95	0.2459	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	0.24292	0.24292	0.24441	0.25186	0.25186	0.25186	0.24888	0.26379	0.26379	0.26379	0.26379
CHE2	0.2623	0.2608	0.2608	0.2608	0.2608	0.2608	0.2608	0.2608	0.2608	0.2608	0.2608	0.25931	0.25931	0.25931	0.25931	0.2608	0.26677	0.26677	0.26677	0.2608	0.26528	0.26528	0.26528	0.26528
POR25	0.26379	0.2623	0.2623	0.2623	0.2623	0.2623	0.2623	0.2623	0.2623	0.2623	0.2623	0.2608	0.2608	0.2608	0.2608	0.2623	0.26826	0.26826	0.26826	0.2623	0.26677	0.26677	0.26677	0.26677
MER64	0.2608	0.25931	0.25931	0.25931	0.25931	0.25931	0.25931	0.25931	0.25931	0.25931	0.25931	0.25782	0.25782	0.25782	0.25782	0.25931	0.26528	0.26528	0.26528	0.25931	0.26379	0.26379	0.26379	0.26379
LAY51	0.26099	0.25943	0.25943	0.25943	0.25943	0.25943	0.25945	0.25945	0.25945	0.25945	0.25945	0.2579	0.2579	0.2579	0.2579	0.258	0.27043	0.27043	0.27043	0.26116	0.26409	0.26409	0.26409	0.26409
LAY52	0.25391	0.25782	0.25782	0.25782	0.25782	0.25782	0.25782	0.25782	0.25782	0.25782	0.25782	0.25633	0.25633	0.25633	0.25633	0.25782	0.26677	0.26677	0.26677	0.2608	0.2623	0.2623	0.2623	0.2623
GIL48B	0.25953	0.25803	0.25803	0.25803	0.25803	0.25803	0.25803	0.25803	0.25803	0.25803	0.25803	0.25654	0.25654	0.25654	0.25654	0.25803	0.26701	0.26701	0.26701	0.26102	0.2625	0.2625	0.2625	0.2625
POR167	0.26528	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.2623	0.2623	0.2623	0.2623	0.26379	0.26975	0.26975	0.26975	0.26379	0.27422	0.27422	0.27422	0.27422
POR99	0.26677	0.26528	0.26528	0.26528	0.26528	0.26528	0.26528	0.26528	0.26528	0.26528	0.26528	0.26379	0.26379	0.26379	0.26379	0.26528	0.27124	0.27124	0.27124	0.26528	0.27571	0.27571	0.27571	0.27571
SEM144E	0.26528	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.26379	0.2623	0.2623	0.2623	0.2623	0.26379	0.26975	0.26975	0.26975	0.26379	0.27422	0.27422	0.27422	0.27422
N.expDes	0.2608	0.25931	0.25931	0.25931	0.25931	0.25931	0.25782	0.25782	0.25782	0.25782	0.25782	0.25633	0.25633	0.25633	0.25633	0.25782	0.2623	0.2623	0.2623	0.25931	0.25633	0.25633	0.25633	0.25633
CHE136	0.2623	0.2608	0.2608	0.2608	0.2608	0.2608	0.25931	0.25931	0.25931	0.25931	0.25931	0.25782	0.25782	0.25782	0.25782	0.25931	0.26379	0.26379	0.26379	0.2608	0.25782	0.25782	0.25782	0.25782
KEM114	0.26826	0.26677	0.26677	0.26677	0.26677	0.26677	0.26528	0.26528	0.26528	0.26528	0.26528	0.26379	0.26379	0.26379	0.26379	0.26528	0.26379	0.26379	0.26379	0.26379	0.25782	0.25782	0.25782	0.25782
LAY85	0.26826	0.26677	0.26677	0.26677	0.26677	0.26677	0.26528	0.26528	0.26528	0.26528	0.26528	0.26379	0.26379	0.26379	0.26379	0.26528	0.27124	0.27124	0.27124	0.26528	0.26528	0.26528	0.26528	0.26528
BES40	0.26623	0.26469	0.26469	0.26469	0.26469	0.26469	0.26317	0.26317	0.26317	0.26317	0.26317	0.26165	0.26165	0.26165	0.26165	0.26317	0.26307	0.26307	0.26307	0.25546	0.25206	0.25206	0.25206	0.25206
BIS58	0.26826	0.26677	0.26677	0.26677	0.26677	0.26677	0.26528	0.26528	0.26528	0.26528	0.26528	0.26379	0.26379	0.26379	0.26379	0.26528	0.26379	0.26379	0.26379	0.25633	0.25186	0.25186	0.25186	0.25186
BIS73	0.26826	0.26677	0.26677	0.26677	0.26677	0.26677	0.26528	0.26528	0.26528	0.26528	0.26528	0.26379	0.26379	0.26379	0.26379	0.26528	0.2623	0.2623	0.2623	0.25484	0.25484	0.25484	0.25484	0.25484
KAT49	0.27869	0.2772	0.2772	0.2772	0.2772	0.2772	0.27571	0.27571	0.27571	0.27571	0.27571	0.27422	0.27422	0.27422	0.27422	0.27571	0.26975	0.26975	0.26975	0.26379	0.26528	0.26528	0.26528	0.26528
NIP50B	0.26528	0.26379	0.26379	0.26379	0.26379	0.26379	0.2623	0.2623	0.2623	0.2623	0.2623	0.2608	0.2608	0.2608	0.2608	0.2623	0.26826	0.26826	0.26826	0.2608	0.26528	0.26528	0.26528	0.26528
GIL70	0.26528	0.26379	0.26379	0.26379	0.26379	0.26379	0.2623	0.2623	0.2623	0.2623	0.2623	0.2608	0.2608	0.2608	0.2608	0.2623	0.26826	0.26826	0.26826	0.2608	0.26528	0.26528	0.26528	0.26528
GIL44	0.25294	0.25127	0.25127	0.25127	0.25127	0.25127	0.25126	0.25126	0.25126	0.25126	0.25126	0.24961	0.24961	0.24961	0.24961	0.25126	0.25738	0.25738	0.25738	0.24926	0.2542	0.2542	0.2542	0.2542
ALO171	0.26379	0.2623	0.2623	0.2623	0.2623	0.2623	0.2608	0.2608	0.2608	0.2608	0.2608	0.25931	0.25931	0.25931	0.25931	0.2608	0.26677	0.26677	0.26677	0.2623	0.2608	0.2608	0.2608	0.2608

Appendix D (continued)

	CHE139A	CHE140C	CHE142	CHE3	CHE168	POR31	SEM144C	SEM160A	KUN148	SEM147	SEM149	SEM151	SEM158A	SEM156	SEM161A	SEM152A	CHE138E	TKS127B	TKR133	BAT7A	MER71	POR30	PTL35	MER16
N.expHam	0.2608	0.25931	0.25931	0.25931	0.25931	0.25931	0.25782	0.25782	0.25782	0.25782	0.25782	0.25633	0.25633	0.25633	0.25633	0.25782	0.2623	0.2623	0.2623	0.25335	0.26528	0.26528	0.26528	0.26528
LAY54	0.23994	0.23845	0.23845	0.23845	0.23845	0.23845	0.23994	0.23994	0.23994	0.23994	0.23994	0.24143	0.24143	0.24143	0.24143	0.24292	0.24143	0.24143	0.24143	0.24143	0.23845	0.23845	0.23845	0.23845
P.roscoff	0.30071	0.29917	0.29917	0.29917	0.29917	0.29917	0.29919	0.29919	0.29919	0.29919	0.29919	0.2992	0.2992	0.2992	0.2992	0.29767	0.30972	0.30972	0.30972	0.31142	0.31236	0.31236	0.31236	0.31236
D.simplex	0.24292	0.24143	0.24143	0.24143	0.24143	0.24143	0.23994	0.23994	0.23994	0.23994	0.23994	0.23845	0.23845	0.23845	0.23845	0.23994	0.23994	0.23994	0.23994	0.23696	0.24888	0.24888	0.24888	0.24888
DipspB	0.24143	0.23994	0.23994	0.23994	0.23994	0.23994	0.23845	0.23845	0.23845	0.23845	0.23845	0.23696	0.23696	0.23696	0.23696	0.23845	0.24143	0.24143	0.24143	0.23547	0.24739	0.24739	0.24739	0.24739
POR33	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.21759	0.22802	0.22802	0.22802	0.22355	0.23547	0.23547	0.23547	0.23547
CHD123	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.21759	0.22802	0.22802	0.22802	0.22355	0.23547	0.23547	0.23547	0.23547
POR169	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.21759	0.22802	0.22802	0.22802	0.22355	0.23547	0.23547	0.23547	0.23547
CHD118	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.21759	0.22802	0.22802	0.22802	0.22355	0.23547	0.23547	0.23547	0.23547
TKS128	0.22206	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21908	0.22951	0.22951	0.22951	0.22504	0.23696	0.23696	0.23696	0.23696
POR94	0.22206	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21908	0.22951	0.22951	0.22951	0.22504	0.23696	0.23696	0.23696	0.23696
CHD121	0.22206	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21908	0.22951	0.22951	0.22951	0.22504	0.23696	0.23696	0.23696	0.23696
GIL42	0.22504	0.22355	0.22355	0.22355	0.22355	0.22355	0.22206	0.22206	0.22206	0.22206	0.22206	0.22057	0.22057	0.22057	0.22057	0.22206	0.23398	0.23398	0.23398	0.22951	0.24143	0.24143	0.24143	0.24143
SEM155	0.21759	0.2161	0.2161	0.2161	0.2161	0.2161	0.21461	0.21461	0.21461	0.21461	0.21461	0.21311	0.21311	0.21311	0.21311	0.21461	0.22951	0.22951	0.22951	0.22504	0.23696	0.23696	0.23696	0.23696
POR24	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.21759	0.22802	0.22802	0.22802	0.22355	0.23547	0.23547	0.23547	0.23547
POR26	0.21908	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.2161	0.2161	0.2161	0.2161	0.21461	0.21461	0.21461	0.21461	0.2161	0.22653	0.22653	0.22653	0.22206	0.23398	0.23398	0.23398	0.23398
LAB80	0.23845	0.23696	0.23696	0.23696	0.23696	0.23696	0.23845	0.23845	0.23845	0.23845	0.23845	0.23696	0.23696	0.23696	0.23696	0.23845	0.24292	0.24292	0.24292	0.22653	0.23845	0.23845	0.23845	0.23845
LAB84	0.23845	0.23696	0.23696	0.23696	0.23696	0.23696	0.23845	0.23845	0.23845	0.23845	0.23845	0.23696	0.23696	0.23696	0.23696	0.23845	0.24292	0.24292	0.24292	0.22653	0.23845	0.23845	0.23845	0.23845
LAB75	0.23845	0.23696	0.23696	0.23696	0.23696	0.23696	0.23845	0.23845	0.23845	0.23845	0.23845	0.23696	0.23696	0.23696	0.23696	0.23845	0.24292	0.24292	0.24292	0.22653	0.23845	0.23845	0.23845	0.23845
RAM65	0.23845	0.23696	0.23696	0.23696	0.23696	0.23696	0.23845	0.23845	0.23845	0.23845	0.23845	0.23696	0.23696	0.23696	0.23696	0.23845	0.24292	0.24292	0.24292	0.22653	0.23845	0.23845	0.23845	0.23845
LAB82	0.23845	0.23696	0.23696	0.23696	0.23696	0.23696	0.23845	0.23845	0.23845	0.23845	0.23845	0.23696	0.23696	0.23696	0.23696	0.23845	0.24292	0.24292	0.24292	0.22653	0.23845	0.23845	0.23845	0.23845
LAB46	0.23845	0.23696	0.23696	0.23696	0.23696	0.23696	0.23845	0.23845	0.23845	0.23845	0.23845	0.23696	0.23696	0.23696	0.23696	0.23845	0.24292	0.24292	0.24292	0.22653	0.23845	0.23845	0.23845	0.23845
RAM47A	0.23994	0.23845	0.23845	0.23845	0.23845	0.23845	0.23994	0.23994	0.23994	0.23994	0.23994	0.23845	0.23845	0.23845	0.23845	0.23994	0.24143	0.24143	0.24143	0.22504	0.23696	0.23696	0.23696	0.23696
CHE141	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.22057	0.22951	0.22951	0.22951	0.21759	0.22951	0.22951	0.22951	0.22951
CHE143C	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.22057	0.22951	0.22951	0.22951	0.21759	0.22951	0.22951	0.22951	0.22951
SAY134	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.22057	0.22951	0.22951	0.22951	0.21759	0.22951	0.22951	0.22951	0.22951
SAY135	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.22057	0.22951	0.22951	0.22951	0.21759	0.22951	0.22951	0.22951	0.22951
CHE139B	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.22057	0.22951	0.22951	0.22951	0.21759	0.22951	0.22951	0.22951	0.22951
CHE137	0.22057	0.21908	0.21908	0.21908	0.21908	0.21908	0.22057	0.22057	0.22057	0.22057	0.22057	0.21908	0.21908	0.21908	0.21908	0.22057	0.22951	0.22951	0.22951	0.21759	0.22951	0.22951	0.22951	0.22951
USA37	0.25633	0.25484	0.25484	0.25484	0.25484	0.25484	0.25484	0.25484	0.25484	0.25484	0.25484	0.25335	0.25335	0.25335	0.25335	0.25484	0.24379	0.24379	0.24379	0.24441	0.25037	0.25037	0.25037	0.25037
MER20	0.33532	0.33383	0.33383	0.33383	0.33383	0.33383	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33383	0.3532	0.3532	0.3532	0.34724	0.35022	0.35022	0.35022	0.35022
GIL45	0.33532	0.33383	0.33383	0.33383	0.33383	0.33383	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33383	0.3532	0.3532	0.3532	0.34724	0.35022	0.35022	0.35022	0.35022
GIL57A	0.33532	0.33383	0.33383	0.33383	0.33383	0.33383	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33383	0.3532	0.3532	0.3532	0.34724	0.35022	0.35022	0.35022	0.35022
GIL89	0.33532	0.33383	0.33383	0.33383	0.33383	0.33383	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33234	0.33383	0.3532	0.3532	0.3532	0.34724	0.35022	0.35022	0.35022	0.35022
RAM47B	0.33383	0.33234	0.33234	0.33234	0.33234	0.33234	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33234	0.35171	0.35171	0.35171	0.34575	0.34873	0.34873	0.34873	0.34873
SEM146	0.33085	0.32936	0.32936	0.32936	0.32936	0.32936	0.32787	0.32787	0.32787	0.32787	0.32787	0.32787	0.32787	0.32787	0.32787	0.32936	0.34873	0.34873	0.34873	0.34277	0.35171	0.35171	0.35171	0.35171
SEM152E	0.33883	0.33234	0.33234	0.33234	0.33234	0.33234	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33234	0.34575	0.34575	0.34575	0.34277	0.34873	0.34873	0.34873	0.34873
SEM160C	0.33883	0.33234	0.33234	0.33234	0.33234	0.33234	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33085	0.33234	0.34575	0.34575	0.34575	0.34277	0.34873	0.34873	0.34873	0.34873
LAB53	0.3532	0.35171	0.35171	0.35171	0.35171	0.35171	0.35022	0.35022	0.35022	0.35022	0.35022	0.35022	0.35022	0.35022	0.35022	0.34873	0.35171	0.35171	0.35171	0.34724	0.34724	0.34724	0.34724	0.34724

Appendix D (continued)

	POR32	MER18	MER13	MER22	MER23	MER11	DAN78	DAN77	TKA109	TKS130	TKA110	MER97	POR96	TKS129	TKS131	BAT43	MER10	MER17	SEM164	SEM166	SEM165	SEM162	SEM163	CHD117A
POR32	N																							
MER18	0.00149	N																						
MER13	0.00149	0	N																					
MER22	0.00149	0	0	N																				
MER23	0.00149	0	0	0	N																			
MER11	0.00154	0	0	0	0	N																		
DAN78	0.12072	0.11923	0.11923	0.11923	0.11923	0.12175	N																	
DAN77	0.12072	0.11923	0.11923	0.11923	0.11923	0.12175	0	N																
TKA109	0.12221	0.12072	0.12072	0.12072	0.12072	0.12325	0.00298	0.00298	N															
TKS130	0.12221	0.12072	0.12072	0.12072	0.12072	0.12325	0.00298	0.00298	0	N														
TKA110	0.12221	0.12072	0.12072	0.12072	0.12072	0.12325	0.00298	0.00298	0	0	N													
MER97	0.12519	0.1237	0.1237	0.1237	0.1237	0.12624	0.02235	0.02235	0.01937	0.01937	0.01937	N												
POR96	0.12519	0.1237	0.1237	0.1237	0.1237	0.12624	0.02235	0.02235	0.01937	0.01937	0.01937	0	N											
TKS129	0.12519	0.1237	0.1237	0.1237	0.1237	0.12624	0.02235	0.02235	0.01937	0.01937	0.01937	0	0	N										
TKS131	0.1237	0.12519	0.12519	0.12519	0.12519	0.12777	0.02385	0.02235	0.02086	0.02086	0.02086	0.00149	0.00149	0.00149	N									
BAT43	0.12221	0.12072	0.12072	0.12072	0.12072	0.12321	0.02235	0.02235	0.01937	0.01937	0.01937	0.00298	0.00298	0.00298	0.00447	N								
MER10	0.1535	0.15201	0.15201	0.15201	0.15201	0.15198	0.11028	0.11028	0.11326	0.11326	0.11326	0.12072	0.12072	0.12072	0.12221	0.12072	N							
MER17	0.1535	0.15201	0.15201	0.15201	0.15201	0.15198	0.11028	0.11028	0.11326	0.11326	0.11326	0.12072	0.12072	0.12072	0.12221	0.12072	0	N						
SEM164	0.14605	0.14456	0.14456	0.14456	0.14456	0.14447	0.11475	0.11475	0.11773	0.11773	0.11773	0.12668	0.12668	0.12668	0.12817	0.1237	0.08197	0.08197	N					
SEM166	0.14605	0.14456	0.14456	0.14456	0.14456	0.14447	0.11475	0.11475	0.11773	0.11773	0.11773	0.12668	0.12668	0.12668	0.12817	0.1237	0.08197	0.08197	0	N				
SEM165	0.14605	0.14456	0.14456	0.14456	0.14456	0.14447	0.11475	0.11475	0.11773	0.11773	0.11773	0.12668	0.12668	0.12668	0.12817	0.1237	0.08197	0.08197	0	0	N			
SEM162	0.14605	0.14456	0.14456	0.14456	0.14456	0.14447	0.11475	0.11475	0.11773	0.11773	0.11773	0.12668	0.12668	0.12668	0.12817	0.1237	0.08197	0.08197	0	0	0	N		
SEM163	0.14307	0.14158	0.14158	0.14158	0.14158	0.14145	0.11177	0.11177	0.11475	0.11475	0.11475	0.1237	0.1237	0.1237	0.12519	0.12072	0.07899	0.07899	0.00298	0.00298	0.00298	0.00298	N	
CHD117A	0.20715	0.20566	0.20566	0.20566	0.20566	0.20682	0.2161	0.2161	0.2161	0.2161	0.2161	0.220257	0.220257	0.220257	0.22206	0.22206	0.21162	0.21162	0.20417	0.20417	0.20417	0.20417	0.20566	N
CHD122A	0.20764	0.2077	0.2077	0.2077	0.2077	0.20898	0.21842	0.21842	0.21841	0.21841	0.21841	0.22296	0.22296	0.22296	0.22293	0.2245	0.21249	0.21249	0.20774	0.20774	0.20774	0.20774	0.20927	0
CHD120	0.20715	0.20566	0.20566	0.20566	0.20566	0.20682	0.2161	0.2161	0.2161	0.2161	0.2161	0.22057	0.22057	0.22057	0.22206	0.22206	0.21162	0.21162	0.20417	0.20417	0.20417	0.20417	0.20566	0
KU1065	0.20566	0.20417	0.20417	0.20417	0.20417	0.20531	0.21461	0.21461	0.21461	0.21461	0.21461	0.21908	0.21908	0.21908	0.22057	0.22057	0.21311	0.21311	0.20268	0.20268	0.20268	0.20268	0.20417	0.00298
BIS62	0.20268	0.20119	0.20119	0.20119	0.20119	0.20199	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20417	0.20417	0.22206	0.22206	0.21908	0.21908	0.21908	0.21908	0.22057	0.09389
BIS63	0.20268	0.20119	0.20119	0.20119	0.20119	0.202	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20268	0.20417	0.20417	0.22206	0.22206	0.21908	0.21908	0.21908	0.21908	0.22057	0.0924
SEM159A	0.19821	0.19672	0.19672	0.19672	0.19672	0.19907	0.1848	0.1848	0.18778	0.18778	0.18778	0.19374	0.19374	0.19374	0.19523	0.19225	0.21013	0.21013	0.20715	0.20715	0.20715	0.20715	0.20864	0.1073
SEM161B	0.19821	0.19672	0.19672	0.19672	0.19672	0.19907	0.1848	0.1848	0.18778	0.18778	0.18778	0.19374	0.19374	0.19374	0.19523	0.19225	0.21013	0.21013	0.20715	0.20715	0.20715	0.20715	0.20864	0.1073
MER14	0.22057	0.22057	0.22057	0.22057	0.22057	0.22181	0.20566	0.20566	0.20566	0.20566	0.20566	0.21162	0.21162	0.21162	0.21162	0.21013	0.20864	0.20864	0.20715	0.20715	0.20715	0.20715	0.20864	0.12817
MER15	0.21908	0.21908	0.21908	0.21908	0.21908	0.22029	0.20417	0.20417	0.20417	0.20417	0.20417	0.21311	0.21311	0.21311	0.21311	0.21162	0.20715	0.20715	0.20864	0.20864	0.20864	0.20864	0.21013	0.12668
CHE1	0.21311	0.21162	0.21162	0.21162	0.21162	0.2113	0.19672	0.19672	0.19672	0.19672	0.19672	0.20119	0.20119	0.20119	0.20268	0.20119	0.21013	0.21013	0.19523	0.19523	0.19523	0.19523	0.19374	0.13562
MesspC	0.20566	0.20417	0.20417	0.20417	0.20417	0.2038	0.18778	0.18778	0.1848	0.1848	0.1848	0.19225	0.19225	0.19225	0.19374	0.19076	0.21013	0.21013	0.20119	0.20119	0.20119	0.20119	0.1997	0.20268
CHE138C	0.20566	0.20417	0.20417	0.20417	0.20417	0.2038	0.18778	0.18778	0.1848	0.1848	0.1848	0.19225	0.19225	0.19225	0.19374	0.19076	0.21013	0.21013	0.20119	0.20119	0.20119	0.20119	0.1997	0.20268
TKS127A	0.20566	0.20417	0.20417	0.20417	0.20417	0.2038	0.18778	0.18778	0.1848	0.1848	0.1848	0.19225	0.19225	0.19225	0.19374	0.19076	0.21013	0.21013	0.20119	0.20119	0.20119	0.20119	0.1997	0.20268
CHE143A	0.20566	0.20417	0.20417	0.20417	0.20417	0.2038	0.18778	0.18778	0.1848	0.1848	0.1848	0.19225	0.19225	0.19225	0.19374	0.19076	0.21013	0.21013	0.20119	0.20119	0.20119	0.20119	0.1997	0.20268
TKR132	0.20715	0.20566	0.20566	0.20566	0.20566	0.20531	0.18927	0.18927	0.18629	0.18629	0.18629	0.19374	0.19374	0.19374	0.19523	0.19225	0.21162	0.21162	0.20268	0.20268	0.20268	0.20268	0.20119	0.20417
POR34	0.20417	0.20268	0.20268	0.20268	0.20268	0.20364	0.18629	0.18629	0.18331	0.18331	0.18331	0.19076	0.19076	0.19076	0.19225	0.18927	0.20715	0.20715	0.19821	0.19821	0.19821	0.19821	0.19672	0.20268
KEM112	0.22653	0.22504	0.22504	0.22504	0.22504	0.22507	0.19225	0.19225	0.19523	0.19523	0.19523	0.20119	0.20119	0.20119	0.20268	0.19821	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20715	0.20119
CHD122B	0.22653	0.22504	0.22504	0.22504	0.22504	0.22507	0.19225	0.19225	0.19523	0.19523	0.19523	0.20119	0.20119	0.20119	0.20268	0.19821	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20715	0.20119
CHD117B	0.22653	0.22504	0.22504	0.22504	0.22504	0.22507	0.19225	0.19225	0.19523	0.19523	0.19523	0.20119	0.20119	0.20119	0.20268	0.19821	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20715	0.20119
KEM115	0.22653	0.22504	0.22504	0.22504	0.22504	0.22507	0.19225	0.19225	0.19523	0.19523	0.19523	0.20119	0.20119	0.20119	0.20268	0.19821	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20715	0.20119
KEM111	0.22653	0.22504	0.22504	0.22504	0.22504	0.22507	0.19225	0.19225	0.19523	0.19523	0.19523	0.20119	0.20119	0.20119	0.20268	0.19821	0.20864	0.20864	0.20864	0.20864	0.20864	0.20864	0.20715	0.20119
KEM113	0.22517	0.22368	0.22368	0.22368	0.22368	0.22368	0.19382	0.19382	0.1968	0.1968	0.1968	0.20279	0.20279	0.20279	0.20428	0.1998	0.21028	0.21028	0.21029	0.21029	0.21029	0.21029	0.20879	0.19986
LEN41	0.21759	0.21908	0.21908	0.21908	0.21908	0.22011	0.19523	0.19523	0.19821	0.19821	0.19821	0.20119	0.20119	0.20119	0.1997	0.20119	0.21759	0.21759	0.21759	0.21759	0.21759	0.21759	0.2161	0.20566
CHE140D	0.22653	0.22504	0.22504	0.22504	0.22504	0.2265	0.1848	0.1848	0.18778	0.18778	0.18778	0.19374	0.19374	0.19374	0.19523	0.19076	0.21908	0.21908	0.21013	0.21013	0.21013	0.21013	0.21013	0.20119
GIL88	0.2161	0.21461	0.21461	0.21461	0.21461	0.21711	0.20715	0.20715	0.20864	0.20864	0.20864	0.21759	0.21759	0.21759	0.21908	0.21461	0.22504	0.22504	0.21461	0.21461	0.21461	0.21461	0.2161	0.19374
GIL57B	0.21461	0.2161	0.2161	0.2161	0.2161	0.21863	0.20864	0.20864	0.21013	0.21013	0.21013	0.21908	0.21908	0.21908	0.21759	0.2161	0.22653	0.22653	0.2161	0.2161	0.2161	0.2161	0.21759	0.19523
CHE140F	0.19225	0.19076	0.19076	0.19076	0.19076	0.19152	0.20119	0.20119	0.20268	0.20268	0.20268	0.20864	0.20864	0.20864	0.21013	0.20715	0.20566	0.20566	0.20268	0.20268	0.20268	0.20268	0.20119	0.20119

Appendix D (continued)

	POR32	MER18	MER13	MER22	MER23	MER11	DAN78	DAN77	TKA109	TKS130	TKA110	MER97	MER96	TKS129	TKS131	BAT43	MER10	MER17	SEM164	SEM166	SEM165	SEM162	SEM163	CHD117A
CHE5	0.19374	0.19225	0.19225	0.19225	0.19225	0.19305	0.20268	0.20268	0.20417	0.20417	0.20417	0.21013	0.21013	0.21013	0.21162	0.20864	0.20715	0.20715	0.20417	0.20417	0.20417	0.20417	0.20268	0.1997
LAY86	0.19225	0.19076	0.19076	0.19076	0.19076	0.19154	0.1997	0.1997	0.20119	0.20119	0.20119	0.20715	0.20715	0.20715	0.20864	0.20566	0.20268	0.20268	0.1997	0.1997	0.1997	0.1997	0.19821	0.20417
MER19	0.18657	0.18507	0.18507	0.18507	0.18507	0.18717	0.19846	0.19846	0.19996	0.19996	0.19996	0.20893	0.20893	0.20893	0.20142	0.20744	0.20302	0.20302	0.20149	0.20149	0.20149	0.20149	0.2	0.19389
MesspD	0.19374	0.19225	0.19225	0.19225	0.19225	0.19449	0.20417	0.20417	0.20566	0.20566	0.20566	0.20864	0.20864	0.20864	0.21013	0.20715	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20268	0.20864
GIL38	0.21461	0.2161	0.2161	0.2161	0.2161	0.21574	0.19374	0.19374	0.19672	0.19672	0.19672	0.1997	0.1997	0.1997	0.19821	0.19672	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20268	0.21162
BAT39	0.21461	0.2161	0.2161	0.2161	0.2161	0.21574	0.19374	0.19374	0.19672	0.19672	0.19672	0.1997	0.1997	0.1997	0.19821	0.19672	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20268	0.21162
GIL56	0.21461	0.2161	0.2161	0.2161	0.2161	0.21574	0.19374	0.19374	0.19672	0.19672	0.19672	0.1997	0.1997	0.1997	0.19821	0.19672	0.20566	0.20566	0.20417	0.20417	0.20417	0.20417	0.20268	0.21162
FUS3	0.21461	0.2161	0.2161	0.2161	0.2161	0.21576	0.19672	0.19672	0.1997	0.1997	0.1997	0.20268	0.20268	0.20268	0.20119	0.1997	0.20566	0.20566	0.20715	0.20715	0.20715	0.20715	0.20566	0.20864
FUS2	0.21461	0.2161	0.2161	0.2161	0.2161	0.21576	0.19672	0.19672	0.1997	0.1997	0.1997	0.20268	0.20268	0.20268	0.20119	0.1997	0.20566	0.20566	0.20715	0.20715	0.20715	0.20715	0.20566	0.20864
NIP66	0.2161	0.21759	0.21759	0.21759	0.21759	0.21726	0.19523	0.19523	0.19821	0.19821	0.19821	0.20119	0.20119	0.20119	0.1997	0.19821	0.20715	0.20715	0.20566	0.20566	0.20566	0.20566	0.20417	0.21013
SEM159D	0.21759	0.21908	0.21908	0.21908	0.21908	0.2188	0.19672	0.19672	0.1997	0.1997	0.1997	0.20268	0.20268	0.20268	0.20119	0.1997	0.20268	0.20268	0.20715	0.20715	0.20715	0.20715	0.20566	0.20864
LAB76	0.22653	0.22504	0.22504	0.22504	0.22504	0.22785	0.20715	0.20715	0.20864	0.20864	0.20864	0.21013	0.21013	0.21013	0.21162	0.20864	0.21461	0.21461	0.20715	0.20715	0.20715	0.20715	0.20566	0.22504
LAB83	0.22653	0.22504	0.22504	0.22504	0.22504	0.22785	0.20715	0.20715	0.20864	0.20864	0.20864	0.21013	0.21013	0.21013	0.21162	0.20864	0.21461	0.21461	0.20715	0.20715	0.20715	0.20715	0.20566	0.22504
LAB81	0.22653	0.22504	0.22504	0.22504	0.22504	0.22785	0.20715	0.20715	0.20864	0.20864	0.20864	0.21013	0.21013	0.21013	0.21162	0.20864	0.21461	0.21461	0.20715	0.20715	0.20715	0.20715	0.20566	0.22504
LAB74	0.22653	0.22504	0.22504	0.22504	0.22504	0.22785	0.20715	0.20715	0.20864	0.20864	0.20864	0.21013	0.21013	0.21013	0.21162	0.20864	0.21461	0.21461	0.20715	0.20715	0.20715	0.20715	0.20566	0.22504
LAB79	0.22653	0.22504	0.22504	0.22504	0.22504	0.22785	0.20715	0.20715	0.20864	0.20864	0.20864	0.21013	0.21013	0.21013	0.21162	0.20864	0.21461	0.21461	0.20715	0.20715	0.20715	0.20715	0.20566	0.22504
LAY55	0.22653	0.22504	0.22504	0.22504	0.22504	0.22785	0.20715	0.20715	0.20864	0.20864	0.20864	0.21013	0.21013	0.21013	0.21162	0.20864	0.21461	0.21461	0.20715	0.20715	0.20715	0.20715	0.20566	0.22504
R.fungi	0.25499	0.25514	0.25514	0.25514	0.25514	0.25444	0.23706	0.23706	0.23854	0.23854	0.23854	0.23849	0.23849	0.23849	0.2833	0.23697	0.25689	0.25689	0.25704	0.25704	0.25704	0.25704	0.25704	0.26469
A. japon	0.27005	0.2701	0.2701	0.2701	0.2701	0.26791	0.2641	0.2641	0.26711	0.26711	0.26711	0.26704	0.26704	0.26704	0.26701	0.26552	0.26111	0.26111	0.27788	0.27788	0.27788	0.27788	0.27635	0.26419
CHE4	0.26641	0.26486	0.26486	0.26486	0.26486	0.26527	0.24815	0.24815	0.24969	0.24969	0.24969	0.25279	0.25279	0.25279	0.25432	0.25127	0.24334	0.24334	0.25101	0.25101	0.25101	0.25101	0.24798	0.26325
MER21	0.26379	0.2623	0.2623	0.2623	0.2623	0.263	0.24739	0.24739	0.24888	0.24888	0.24888	0.25186	0.25186	0.25186	0.25335	0.25037	0.24292	0.24292	0.25037	0.25037	0.25037	0.25037	0.24739	0.2608
MER91	0.26379	0.2623	0.2623	0.2623	0.2623	0.263	0.24739	0.24739	0.24888	0.24888	0.24888	0.25186	0.25186	0.25186	0.25335	0.25037	0.24292	0.24292	0.25037	0.25037	0.25037	0.25037	0.24739	0.2608
CHE6	0.26379	0.2623	0.2623	0.2623	0.2623	0.263	0.24739	0.24739	0.24888	0.24888	0.24888	0.25186	0.25186	0.25186	0.25335	0.25037	0.24292	0.24292	0.25037	0.25037	0.25037	0.25037	0.24739	0.2608
POR27	0.26379	0.2623	0.2623	0.2623	0.2623	0.263	0.24739	0.24739	0.24888	0.24888	0.24888	0.25186	0.25186	0.25186	0.25335	0.25037	0.24292	0.24292	0.25037	0.25037	0.25037	0.25037	0.24739	0.2608
POR100	0.26379	0.2623	0.2623	0.2623	0.2623	0.263	0.24739	0.24739	0.24888	0.24888	0.24888	0.25186	0.25186	0.25186	0.25335	0.25037	0.24292	0.24292	0.25037	0.25037	0.25037	0.25037	0.24739	0.2608
MER95	0.26379	0.2623	0.2623	0.2623	0.2623	0.26429	0.24739	0.24739	0.24888	0.24888	0.24888	0.25186	0.25186	0.25186	0.25037	0.25037	0.24292	0.24292	0.25037	0.25037	0.25037	0.25037	0.24739	0.26379
CHE2	0.26528	0.26677	0.26677	0.26677	0.26677	0.26755	0.25931	0.25931	0.2623	0.2623	0.2623	0.26677	0.26677	0.26677	0.26528	0.26528	0.27571	0.27571	0.25931	0.25931	0.25931	0.25931	0.2608	0.25474
POR25	0.26677	0.26826	0.26826	0.26826	0.26826	0.26778	0.2608	0.2608	0.26379	0.26379	0.26379	0.26826	0.26826	0.26826	0.2667	0.26677	0.2772	0.2772	0.2608	0.2608	0.2608	0.2608	0.2623	0.25335
MER64	0.26379	0.26528	0.26528	0.26528	0.26528	0.26603	0.25782	0.25782	0.2608	0.2608	0.2608	0.26528	0.26528	0.26528	0.26379	0.26379	0.27422	0.27422	0.2608	0.2608	0.2608	0.2608	0.2623	0.25633
LAY51	0.26409	0.26563	0.26563	0.26563	0.26563	0.26603	0.25971	0.25971	0.26282	0.26282	0.26282	0.27064	0.27064	0.27064	0.2691	0.26913	0.27314	0.27314	0.25941	0.25941	0.25941	0.25941	0.26098	0.25189
LAY52	0.2623	0.26379	0.26379	0.26379	0.26379	0.26452	0.25931	0.25931	0.2623	0.2623	0.2623	0.26975	0.26975	0.26975	0.26826	0.26826	0.27273	0.27273	0.25931	0.25931	0.25931	0.25931	0.2608	0.25335
GIL48B	0.2625	0.26399	0.26399	0.26399	0.26399	0.26473	0.25954	0.25954	0.26252	0.26252	0.26252	0.26996	0.26996	0.26996	0.26847	0.26846	0.27299	0.27299	0.25957	0.25957	0.25957	0.25957	0.26106	0.25356
POR167	0.27422	0.27571	0.27571	0.27571	0.27571	0.27667	0.25931	0.25931	0.2623	0.2623	0.2623	0.26379	0.26379	0.26379	0.2623	0.2623	0.27422	0.27422	0.25633	0.25633	0.25633	0.25633	0.25782	0.25484
POR99	0.27571	0.2772	0.2772	0.2772	0.2772	0.27689	0.2608	0.2608	0.26379	0.26379	0.26379	0.26528	0.26528	0.26528	0.26379	0.26379	0.27571	0.27571	0.25782	0.25782	0.25782	0.25782	0.25931	0.25335
SEM144E	0.27422	0.27571	0.27571	0.27571	0.27571	0.27666	0.25931	0.25931	0.22623	0.22623	0.22623	0.26379	0.26379	0.26379	0.2623	0.2623	0.27422	0.27422	0.25633	0.25633	0.25633	0.25633	0.25782	0.245484
N.expDes	0.25633	0.25633	0.25633	0.25633	0.25633	0.25834	0.23696	0.23696	0.23547	0.23547	0.23547	0.23547	0.23547	0.23547	0.23547	0.23547	0.24441	0.24441	0.25335	0.25335	0.25335	0.25335	0.25037	0.23249
CHE136	0.25782	0.25782	0.25782	0.25782	0.25782	0.25851	0.23845	0.23845	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.2459	0.2459	0.25484	0.25484	0.25484	0.25484	0.25186	0.231
KEM114	0.25782	0.25782	0.25782	0.25782	0.25782	0.25969	0.24292	0.24292	0.24292	0.24292	0.24292	0.24441	0.24441	0.24441	0.24441	0.24441	0.25186	0.25186	0.25335	0.25335	0.25335	0.25335	0.25186	0.24441
LAY85	0.26528	0.26528	0.26528	0.26528	0.26528	0.26435	0.2459	0.2459	0.224739	0.224739	0.224739	0.25186	0.25186	0.25186	0.25186	0.25186	0.25186	0.25186	0.25782	0.25782	0.25782	0.25782	0.25484	0.23696
BES40	0.25206	0.25221	0.25221	0.25221	0.25221	0.25306	0.23551	0.23551	0.23701	0.23701	0.23701	0.23848	0.23848	0.23848	0.23833	0.23848	0.25838	0.25838	0.25708	0.25708	0.25708	0.25708	0.25401	0.23107
BIS58	0.25186	0.25335	0.25335	0.25335	0.25335	0.25524	0.23994	0.23994	0.23845	0.23845	0.23845	0.23994	0.23994	0.23994	0.23845	0.23994	0.25633	0.25633	0.25484	0.25484	0.25484	0.25484	0.25186	0.231
BIS73	0.25484	0.25484	0.25484	0.25484	0.25484	0.25538	0.23547	0.23547	0.23696	0.23696	0.23696	0.23845	0.23845	0.23845	0.23845	0.23845	0.25335	0.25335	0.25037	0.25037	0.25037	0.25037	0.24739	0.23249
KAT49	0.26528	0.26528	0.26528	0.26528	0.26528	0.26591	0.23994	0.23994	0.24143	0.24143	0.24143	0.23696	0.23696	0.23696	0.23696	0.23696	0.25335	0.25335	0.25931	0.25931	0.25931	0.25931	0.25633	0.23547
NIP50B	0.26528	0.26528	0.26528	0.26528	0.26528	0.26592	0.24441	0.24441	0.2459	0.2459	0.2459	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	0.25186	0.25186	0.25186	0.25186	0.25186	0.23547
GIL70	0.26528	0.26528	0.26528	0.26528	0.26528	0.26592	0.24441	0.24441	0.2459	0.2459	0.2459	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	0.25186	0.25186	0.25186	0.25186	0.25186	0.23547
GIL44	0.2542	0.25422	0.25422	0.25422	0.25422	0.25486	0.23126	0.23126	0.2329	0.2329	0.2329	0.23112	0.23112	0.23112	0.2311	0.23117	0.22905	0.22905	0.2377	0.2377	0.2377	0.2377	0.23769	0.22431
ALO171	0.2608	0.2608	0.2608	0.2608	0.2608	0.26151	0.24292	0.24292	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24292	0.24292	24888	24888	24888	24888	0.24888	0.23547
N.expHam	0.26528	0.26528	0.26528	0.26528	0.26528	0.26741	0.23845	0.23845	0.23845	0.23845	0.23845	0.24143	0.24143	0.24143	0.24143	0.24143	0.25286	0.25286	0.24441	0.24441	0.24441	0.24441	0.24441	0.23547
LAY54	0.23845	0.23994	0.23994	0.23994	0.23994	0.24271	0.22802	0.22802	0.22951	0.22951	0.22951	0.22653	0.22653	0.22653	0.22594	0.22653	0.24441	0.24441	0.25484	0.25484	0.25484	0.25484	0.25186	0.2608
P.roscoff	0.31236	0.31253	0.31253	0.31253	0.31253	0.31271	0.29924	0.29924	0.29923	0.29923	0.29923	0.29454	0.29454	0.29454	0.29443	0.29301	0.30821	0.30821	0.29629	0.29629	0.29629	0.29629	0.29473	0.29779
D.simplex	0.24888	0.25037	0.25037	0.25037	0.25037	0.25047	0.231	0.231	0.23249	0.23249	0.23249	0.23547	0.23547	0.23547	0.23398	0.23398	0.25037	0.25037	0.2459	0.2459	0.2459	0.2459	0.24441	0.28316

Appendix D (continued)

	POR32	MER18	MER13	MER22	MER23	MER11	DAN78	DAN77	TKA109	TKS130	TKA110	MER97	MER96	TKS129	TKS131	BAT43	MER10	MER17	SEM164	SEM166	SEM165	SEM162	SEM163	CHD117A
DipspB	0.24739	0.24888	0.24888	0.24888	0.24888	0.24897	0.22951	0.22951	0.231	0.231	0.231	0.23398	0.23398	0.23398	0.23249	0.23249	0.24888	0.24888	0.24441	0.24441	0.24441	0.24441	0.24292	0.28167
POR33	0.23547	0.23398	0.23398	0.23398	0.23398	0.23716	0.22504	0.22504	0.22504	0.22504	0.22504	0.22653	0.22653	0.22653	0.22802	0.22504	0.23547	0.23547	0.23845	0.23845	0.23845	0.23845	0.23845	0.25931
CHD123	0.23547	0.23398	0.23398	0.23398	0.23398	0.23716	0.22504	0.22504	0.22504	0.22504	0.22504	0.22653	0.22653	0.22653	0.22802	0.22504	0.23547	0.23547	0.23845	0.23845	0.23845	0.23845	0.23845	0.25931
POR169	0.23547	0.23398	0.23398	0.23398	0.23398	0.23716	0.22504	0.22504	0.22504	0.22504	0.22504	0.22653	0.22653	0.22653	0.22802	0.22504	0.23547	0.23547	0.23845	0.23845	0.23845	0.23845	0.23845	0.25931
CHD118	0.23547	0.23398	0.23398	0.23398	0.23398	0.23716	0.22504	0.22504	0.22504	0.22504	0.22504	0.22653	0.22653	0.22653	0.22802	0.22504	0.23547	0.23547	0.23845	0.23845	0.23845	0.23845	0.23845	0.25931
TKS128	0.23696	0.23547	0.23547	0.23547	0.23547	0.23867	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22802	0.22802	0.22951	0.22653	0.23696	0.23696	0.23994	0.23994	0.23994	0.23994	0.23994	0.2608
POR94	0.23696	0.23547	0.23547	0.23547	0.23547	0.23739	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22802	0.22802	0.22951	0.22653	0.23696	0.23696	0.23994	0.23994	0.23994	0.23994	0.23994	0.25782
CHD121	0.23696	0.23547	0.23547	0.23547	0.23547	0.23739	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22802	0.22802	0.22951	0.22653	0.23696	0.23696	0.23994	0.23994	0.23994	0.23994	0.23994	0.25782
GIL42	0.24143	0.23994	0.23994	0.23994	0.23994	0.24194	0.231	0.231	0.231	0.231	0.231	0.23249	0.23249	0.23249	0.23398	0.231	0.24143	0.24143	0.24441	0.24441	0.24441	0.24441	0.24441	0.2623
SEM155	0.23696	0.23547	0.23547	0.23547	0.23547	0.23866	0.22355	0.22355	0.22355	0.22355	0.22355	0.22504	0.22504	0.22504	0.26653	0.22355	0.23398	0.23398	0.23696	0.23696	0.23696	0.23696	0.23696	0.2608
POR24	0.23547	0.23398	0.23398	0.23398	0.23398	0.23588	0.22206	0.22206	0.22206	0.22206	0.22206	0.22355	0.22355	0.22355	0.22504	0.22206	0.23547	0.23547	0.23547	0.23547	0.23547	0.23547	0.23547	0.25782
POR26	0.23398	0.23249	0.23249	0.23249	0.23249	0.23564	0.22057	0.22057	0.22057	0.22057	0.22057	0.22206	0.22206	0.22206	0.22355	0.22057	0.23398	0.23398	0.23398	0.23398	0.23398	0.23398	0.23398	0.25931
LAB80	0.23845	0.23994	0.23994	0.23994	0.23994	0.24142	0.22206	0.22206	0.22355	0.22355	0.22355	0.23249	0.23249	0.23249	0.231	0.23398	0.23845	0.23845	0.23398	0.23398	0.23398	0.23398	0.23249	0.25931
LAB84	0.23845	0.23994	0.23994	0.23994	0.23994	0.24142	0.22206	0.22206	0.22355	0.22355	0.22355	0.23249	0.23249	0.23249	0.231	0.23398	0.23845	0.23845	0.23398	0.23398	0.23398	0.23398	0.23249	0.25931
LAB75	0.23845	0.23994	0.23994	0.23994	0.23994	0.24142	0.22206	0.22206	0.22355	0.22355	0.22355	0.23249	0.23249	0.23249	0.231	0.23398	0.23845	0.23845	0.23398	0.23398	0.23398	0.23398	0.23249	0.25931
RAM65	0.23845	0.23994	0.23994	0.23994	0.23994	0.24142	0.22206	0.22206	0.22355	0.22355	0.22355	0.23249	0.23249	0.23249	0.231	0.23398	0.23845	0.23845	0.23398	0.23398	0.23398	0.23398	0.23249	0.25931
LAB82	0.23845	0.23994	0.23994	0.23994	0.23994	0.24142	0.22206	0.22206	0.22355	0.22355	0.22355	0.23249	0.23249	0.23249	0.231	0.23398	0.23845	0.23845	0.23398	0.23398	0.23398	0.23398	0.23249	0.25931
LAB46	0.23845	0.23994	0.23994	0.23994	0.23994	0.24142	0.22206	0.22206	0.22355	0.22355	0.22355	0.23249	0.23249	0.23249	0.231	0.23398	0.23845	0.23845	0.23398	0.23398	0.23398	0.23398	0.23249	0.25931
RAM47A	0.23696	0.23845	0.23845	0.23845	0.23845	0.23991	0.22355	0.22355	0.22504	0.22504	0.22504	0.23398	0.23398	0.23398	0.23249	0.23547	0.23994	0.23994	0.23547	0.23547	0.23547	0.23547	0.23398	0.25782
CHE141	0.22951	0.22802	0.22802	0.22802	0.22802	0.228	0.22504	0.22504	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22355	0.24143	0.24143	0.23547	0.23547	0.23547	0.23547	0.23398	0.26975
CHE143C	0.22951	0.22802	0.22802	0.22802	0.22802	0.228	0.22504	0.22504	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22355	0.24143	0.24143	0.23547	0.23547	0.23547	0.23547	0.23398	0.26975
SAY134	0.22951	0.22802	0.22802	0.22802	0.22802	0.228	0.22504	0.22504	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22355	0.24143	0.24143	0.23547	0.23547	0.23547	0.23547	0.23398	0.26975
SAY135	0.22951	0.22802	0.22802	0.22802	0.22802	0.228	0.22504	0.22504	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22355	0.24143	0.24143	0.23547	0.23547	0.23547	0.23547	0.23398	0.26975
CHE139B	0.22951	0.22802	0.22802	0.22802	0.22802	0.228	0.22504	0.22504	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22355	0.24143	0.24143	0.23547	0.23547	0.23547	0.23547	0.23398	0.26975
CHE137	0.22951	0.22802	0.22802	0.22802	0.22802	0.228	0.22504	0.22504	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22802	0.22355	0.24143	0.24143	0.23547	0.23547	0.23547	0.23547	0.23398	0.26975
USA37	0.25037	0.25037	0.25037	0.25037	0.25037	0.25157	0.25782	0.25782	0.22653	0.22653	0.22653	0.25335	0.25335	0.25335	0.25335	0.25633	0.26528	0.26528	0.25633	0.25633	0.25633	0.25633	0.25633	0.27422
MER20	0.35022	0.34873	0.34873	0.34873	0.34873	0.35069	0.34128	0.34128	0.34426	0.34426	0.34426	0.35171	0.35171	0.35171	0.3532	0.35022	0.34128	0.34128	0.35768	0.35768	0.35768	0.35768	0.35768	0.35917
GIL45	0.35022	0.34873	0.34873	0.34873	0.34873	0.35069	0.34128	0.34128	0.34426	0.34426	0.34426	0.35171	0.35171	0.35171	0.3532	0.35022	0.34128	0.34128	0.35768	0.35768	0.35768	0.35768	0.35768	0.35917
GIL57A	0.35022	0.34873	0.34873	0.34873	0.34873	0.35069	0.34128	0.34128	0.34426	0.34426	0.34426	0.35171	0.35171	0.35171	0.3532	0.35022	0.34128	0.34128	0.35768	0.35768	0.35768	0.35768	0.35768	0.35917
GIL89	0.35022	0.34873	0.34873	0.34873	0.34873	0.35069	0.34128	0.34128	0.34426	0.34426	0.34426	0.35171	0.35171	0.35171	0.3532	0.35022	0.34128	0.34128	0.35768	0.35768	0.35768	0.35768	0.35768	0.35917
RAM47B	0.34873	0.34724	0.34724	0.34724	0.34724	0.34917	0.33979	0.33979	0.34277	0.34277	0.34277	0.35022	0.35022	0.35022	0.35171	0.34873	0.33979	0.33979	0.35618	0.35618	0.35618	0.35618	0.35618	0.35768
SEM146	0.35171	0.35022	0.35022	0.35022	0.35022	0.35219	0.33681	0.33681	0.33979	0.33979	0.33979	0.34724	0.34724	0.34724	0.34873	0.34575	0.33681	0.33681	0.35469	0.35469	0.35469	0.35469	0.3532	0.35469
SEM152E	0.34873	0.34724	0.34724	0.34724	0.34724	0.34906	0.3383	0.3383	0.3383	0.3383	0.3383	0.34575	0.34575	0.34575	0.34724	0.34426	0.34724	0.34724	0.34575	0.34575	0.34575	0.34575	0.34873	0.3532
SEM160C	0.34873	0.34724	0.34724	0.34724	0.34724	0.34904	0.3383	0.3383	0.3383	0.3383	0.3383	0.34426	0.34426	0.34426	0.34575	0.34277	0.35022	0.35022	0.34575	0.34575	0.34575	0.34575	0.34873	0.35469
LAB53	0.34724	0.34575	0.34575	0.34575	0.34575	0.3487	0.34128	0.34128	0.34128	0.34128	0.34128	0.33979	0.33979	0.33979	0.34128	0.34128	0.34575	0.34575	0.36215	0.36215	0.36215	0.36215	0.36364	0.35022

Appendix D (continued)

	CHD122A	CHD120	KU1065	BIS62	BIS63	SEM159A	SEM161B	MER14	MER15	CHE1	MesspC	CHE138C	TKS127A	CHE143A	TKR132	POR34	KEM112	CHD122B	CHD117B	KEM115	KEM111	KEM113	LEN41	CHE140D
CHD122A	N																							
CHD120	0	N																						
KU1065	0.00304	0.00298	N																					
BIS62	0.09464	0.09389	0.09538	N																				
BIS63	0.0931	0.0924	0.09389	0.00149	N																			
SEM159A	0.10995	0.1073	0.10581	0.12221	0.12072	N																		
SEM161B	0.10995	0.1073	0.10581	0.12221	0.12072	0	N																	
MER14	0.12983	0.12817	0.12817	0.15052	0.14903	0.13413	0.13413	N																
MER15	0.1283	0.12668	0.12668	0.14903	0.14754	0.13264	0.13264	0.00149	N															
CHE1	0.13893	0.13562	0.13264	0.13562	0.13413	0.13264	0.13264	0.12966	0.12817	N														
MesspC	0.2016	0.20268	0.20119	0.19225	0.19225	0.18331	0.18331	0.20119	0.1997	0.20864	N													
CHE138C	0.2016	0.20268	0.20119	0.19225	0.19225	0.18331	0.18331	0.20119	0.1997	0.20864	0	N												
TKS127A	0.2016	0.20268	0.20119	0.19225	0.19225	0.18331	0.18331	0.20119	0.1997	0.20864	0	0	N											
CHE143A	0.2016	0.20268	0.20119	0.19225	0.19225	0.18331	0.18331	0.20119	0.1997	0.20864	0	0	0	N										
TKR132	0.20314	0.20417	0.20268	0.19374	0.19374	0.1848	0.1848	0.20268	0.20119	0.21013	0.00149	0.00149	0.00149	0.00149	N									
POR34	0.2016	0.20268	0.20119	0.18927	0.18927	0.18033	0.18033	0.19821	0.19672	0.20566	0.00298	0.00298	0.00298	0.00298	0.00447	N								
KEM112	0.2003	0.20119	0.1997	0.20119	0.20119	0.18629	0.18629	0.22206	0.22057	0.20864	0.17139	0.17139	0.17139	0.17139	0.17288	0.17288	N							
CHD122B	0.2003	0.20119	0.1997	0.20119	0.20119	0.18629	0.18629	0.22206	0.22057	0.20864	0.17139	0.17139	0.17139	0.17139	0.17288	0.17288	0	N						
CHD117B	0.2003	0.20119	0.1997	0.20119	0.20119	0.18629	0.18629	0.22206	0.22057	0.20864	0.17139	0.17139	0.17139	0.17139	0.17288	0.17288	0	0	N					
KEM115	0.2003	0.20119	0.1997	0.20119	0.20119	0.18629	0.18629	0.22206	0.22057	0.20864	0.17139	0.17139	0.17139	0.17139	0.17288	0.17288	0	0	0	N				
KEM111	0.2003	0.20119	0.1997	0.20119	0.20119	0.18629	0.18629	0.22206	0.22057	0.20864	0.17139	0.17139	0.17139	0.17139	0.17288	0.17288	0	0	0	0	N			
KEM113	0.19895	0.19986	0.19837	0.19984	0.19984	0.1879	0.1879	0.2237	0.22221	0.20722	0.17296	0.17296	0.17296	0.17296	0.17446	0.17445	0.00149	0.00149	0.00149	0.00149	0.00149	N		
LEN41	0.20332	0.20566	0.20417	0.19821	0.19821	0.19225	0.19225	0.21908	0.21759	0.21013	0.17437	0.17437	0.17437	0.17437	0.17586	0.17288	0.03875	0.03875	0.03875	0.03875	0.03875	0.03727	N	
CHE140D	0.20166	0.20119	0.1997	0.1997	0.1997	0.17437	0.17437	0.21461	0.21311	0.20566	0.18182	0.18182	0.18182	0.18182	0.18331	0.18331	0.0901	0.0901	0.0901	0.0901	0.0901	0.09241	0.08495	N
GIL88	0.19401	0.19374	0.19523	0.20566	0.20417	0.17288	0.17288	0.21311	0.21162	0.20268	0.17735	0.17735	0.17735	0.17735	0.17884	0.17437	0.14605	0.14605	0.14605	0.14605	0.14605	0.14617	0.14903	0.14456
GIL57B	0.19387	0.19523	0.19672	0.20715	0.20566	0.17437	0.17437	0.21311	0.21162	0.20417	0.17884	0.17884	0.17884	0.17884	0.18033	0.17586	0.14754	0.14754	0.14754	0.14754	0.14754	0.14766	0.14754	0.14605
CHE140F	0.1986	0.20119	0.1997	0.20715	0.20715	0.19225	0.19225	0.20119	0.1997	0.20715	0.18182	0.18182	0.18182	0.18182	0.18331	0.17884	0.1997	0.1997	0.1997	0.1997	0.1997	0.2013	0.20119	0.20417
CHE5	0.19706	0.1997	0.19821	0.20566	0.20566	0.19076	0.19076	0.1997	0.19821	0.20566	0.18331	0.18331	0.18331	0.18331	0.1848	0.18033	0.19821	0.19821	0.19821	0.19821	0.19821	0.19981	0.1997	0.20268
LAY86	0.20164	0.20417	0.20268	0.21013	0.21013	0.19523	0.19523	0.20268	0.20119	0.21162	0.18331	0.18331	0.18331	0.18331	0.1848	0.18033	0.20119	0.20119	0.20119	0.20119	0.20119	0.2028	0.20268	0.20566
MER19	0.19108	0.19389	0.1924	0.20744	0.20744	0.18496	0.18496	0.20446	0.20296	0.21048	0.18347	0.18347	0.18347	0.18347	0.18496	0.18049	0.19542	0.19542	0.19542	0.19542	0.19542	0.19703	0.19988	0.19987
MesspD	0.2062	0.20864	0.21013	0.21461	0.21461	0.19374	0.19374	0.20417	0.20268	0.21311	0.18778	0.18778	0.18778	0.18778	0.18927	0.1848	0.20119	0.20119	0.20119	0.20119	0.20119	0.20279	0.20268	0.20566
GIL38	0.2093	0.21162	0.21311	0.21013	0.21162	0.1997	0.1997	0.2161	0.21461	0.21162	0.21461	0.21461	0.21461	0.21461	0.2161	0.21162	0.21311	0.21311	0.21311	0.21311	0.21311	0.21476	0.21162	0.22653
BAT39	0.2093	0.21162	0.21311	0.21013	0.21162	0.1997	0.1997	0.2161	0.21461	0.21162	0.21461	0.21461	0.21461	0.21461	0.2161	0.21162	0.21311	0.21311	0.21311	0.21311	0.21311	0.21476	0.21162	0.22653
GIL56	0.2093	0.21162	0.21311	0.21013	0.21162	0.1997	0.1997	0.2161	0.21461	0.21162	0.21461	0.21461	0.21461	0.21461	0.2161	0.21162	0.21311	0.21311	0.21311	0.21311	0.21311	0.21476	0.21162	0.22653
FUS3	0.20625	0.20864	0.21013	0.21013	0.21162	0.20268	0.20268	0.21908	0.21759	0.21461	0.2161	0.2161	0.2161	0.2161	0.21759	0.21311	0.21311	0.21311	0.21311	0.21311	0.21311	0.21476	0.21162	0.22653
FUS2	0.20625	0.20864	0.21013	0.21013	0.21162	0.20268	0.20268	0.21908	0.21759	0.21461	0.2161	0.2161	0.2161	0.2161	0.21759	0.21311	0.21311	0.21311	0.21311	0.21311	0.21311	0.21476	0.21162	0.22653
NIP66	0.20777	0.21013	0.21162	0.21162	0.21311	0.20119	0.20119	0.21759	0.2161	0.21311	0.2161	0.2161	0.2161	0.2161	0.21759	0.21311	0.21162	0.21162	0.21162	0.21162	0.21162	0.21327	0.21013	0.22504
SEM159D	0.20625	0.20864	0.21013	0.21013	0.21162	0.20268	0.20268	0.2161	0.21461	0.21461	0.2161	0.2161	0.2161	0.2161	0.21759	0.21311	0.21311	0.21311	0.21311	0.21311	0.21311	0.21476	0.21162	0.22653
LAB76	0.22776	0.22504	0.22355	0.2161	0.21759	0.21013	0.21013	0.22951	0.22802	0.22057	0.20715	0.20715	0.20715	0.20715	0.20864	0.20566	0.19523	0.19523	0.19523	0.19523	0.19523	0.19672	0.19821	0.1997
LAB83	0.22776	0.22504	0.22355	0.2161	0.21759	0.21013	0.21013	0.22951	0.22802	0.22057	0.20715	0.20715	0.20715	0.20715	0.20864	0.20566	0.19523	0.19523	0.19523	0.19523	0.19523	0.19672	0.19821	0.1997
LAB81	0.22776	0.22504	0.22355	0.2161	0.21759	0.21013	0.21013	0.22951	0.22802	0.22057	0.20715	0.20715	0.20715	0.20715	0.20864	0.20566	0.19523	0.19523	0.19523	0.19523	0.19523	0.19672	0.19821	0.1997
LAB74	0.22776	0.22504	0.22355	0.2161	0.21759	0.21013	0.21013	0.22951	0.22802	0.22057	0.20715	0.20715	0.20715	0.20715	0.20864	0.20566	0.19523	0.19523	0.19523	0.19523	0.19523	0.19672	0.19821	0.1997
LAB79	0.22776	0.22504	0.22355	0.2161	0.21759	0.21013	0.21013	0.22951	0.22802	0.22057	0.20715	0.20715	0.20715	0.20715	0.20864	0.20566	0.19523	0.19523	0.19523	0.19523	0.19523	0.19672	0.19821	0.1997
LAY55	0.22776	0.22504	0.22355	0.2161	0.21759	0.21013	0.21013	0.22951	0.22802	0.22057	0.20715	0.20715	0.20715	0.20715	0.20864	0.20566	0.19523	0.19523	0.19523	0.19523	0.19523	0.19672	0.19821	0.1997
R.fungi	0.26369	0.26469	0.26623	0.25544	0.25546	0.23565	0.23565	0.24483	0.24636	0.26175	0.24193	0.24193	0.24193	0.24193	0.24345	0.24329	0.25403	0.25403	0.25403	0.25403	0.25403	0.25552	0.24737	0.25715
A. japon	0.25944	0.26419	0.2627	0.26269	0.26271	0.25802	0.25802	0.2657	0.26419	0.25973	0.26591	0.26591	0.26591	0.26591	0.26743	0.26427	0.24454	0.24454	0.24454	0.24454	0.24454	0.24602	0.24699	0.26427
CHE4	0.26681	0.26325	0.2617	0.25418	0.2557	0.24058	0.24058	0.26772	0.26619	0.25098	0.26207	0.26207	0.26207	0.26207	0.26359	0.26048	0.26662	0.26662	0.26662	0.26662	0.26662	0.26813	0.27871	0.2787
MER21	0.26419	0.2608	0.25931	0.25484	0.25633	0.24143	0.24143	0.26826	0.26677	0.25186	0.2623	0.2623	0.2623	0.2623	0.26379	0.2623	0.26528	0.26528	0.26528	0.26528	0.26528	0.26677	0.27869	0.27571
MER91	0.26419	0.2608	0.25931	0.25484	0.25633	0.24143	0.24143	0.26826	0.26677	0.25186	0.2623	0.2623	0.2623	0.2623	0.26379	0.2623	0.26528	0.26528	0.26528	0.26528	0.26528	0.26677	0.27869	0.27571
CHE6	0.26419	0.2608	0.25931	0.25484	0.25633	0.24143	0.24143	0.26826	0.26677	0.25186	0.2623	0.2623	0.2623	0.2623	0.26379	0.2623	0.26528	0.26528	0.26528	0.26528	0.26528	0.26677	0.27869	0.27571
POR27	0.26419	0.2608	0.25931	0.25484	0.25633	0.24143	0.24143	0.26826	0.26677	0.25186	0.2623	0.2623	0.2623	0.2623	0.26379	0.2623	0.26528	0.26528	0.26528	0.26528	0.26528	0.26677	0.27869	0.27571

Appendix D (continued)

	CHD122A	CHD120	KU1065	BIS62	BIS63	SEM159A	SEM161B	MER14	MER15	CHE1	MesspC	CHE138C	TKS127A	CHE143A	TKR132	POR34	KEM112	CHD122B	CHD117B	KEM115	KEM111	KEM113	LEN41	CHE140D
POR100	0.26419	0.2608	0.25931	0.25484	0.25633	0.24143	0.24143	0.26826	0.26677	0.25186	0.2623	0.2623	0.2623	0.2623	0.26379	0.2623	0.26528	0.26528	0.26528	0.26528	0.26528	0.26677	0.27869	0.27571
MER95	0.26566	0.26379	0.2623	0.25484	0.25633	0.24143	0.24143	0.26677	0.26528	0.25186	0.26528	0.26528	0.26528	0.26528	0.26677	0.2623	0.26826	0.26826	0.26826	0.26826	0.26826	0.26975	0.27571	0.27869
CHE2	0.25648	0.25484	0.25335	0.26379	0.26528	0.22951	0.22951	0.24441	0.2459	0.25186	0.2608	0.2608	0.2608	0.2608	0.2623	0.2608	0.25335	0.25335	0.25335	0.25335	0.25335	0.25484	0.25782	0.25335
POR25	0.25495	0.25335	0.25186	0.26528	0.26677	0.231	0.231	0.2459	0.24739	0.25335	0.25931	0.25931	0.25931	0.25931	0.2608	0.2623	0.25186	0.25186	0.25186	0.25186	0.25186	0.25335	0.25931	0.25186
MER64	0.25802	0.25633	0.25484	0.26528	0.26677	0.231	0.231	0.2459	0.24739	0.25335	0.2623	0.2623	0.2623	0.2623	0.26379	0.2623	0.25484	0.25484	0.25484	0.25484	0.25484	0.25633	0.25931	0.25484
LAY51	0.25353	0.25189	0.2503	0.26135	0.26287	0.22888	0.22888	0.24526	0.24372	0.2519	0.25949	0.25949	0.25949	0.25949	0.26102	0.26102	0.25212	0.25212	0.25212	0.25212	0.25212	0.25362	0.25814	0.2502
LAY52	0.25497	0.25335	0.25186	0.2623	0.26379	0.22802	0.22802	0.24739	0.2459	0.25037	0.25782	0.25782	0.25782	0.25782	0.25931	0.25782	0.25186	0.25186	0.25186	0.25186	0.25186	0.25352	0.25633	0.25186
GIL48B	0.25519	0.25356	0.25207	0.2623	0.264	0.22817	0.22817	0.24763	0.24614	0.2506	0.25806	0.25806	0.25806	0.25806	0.25955	0.25806	0.25203	0.25203	0.25203	0.25203	0.25203	0.25352	0.25651	0.25208
POR167	0.25649	0.25484	0.25335	0.26379	0.26258	0.23696	0.23696	0.25186	0.25335	0.25484	0.25782	0.25782	0.25782	0.25782	0.25931	0.25782	0.25186	0.25186	0.25186	0.25186	0.25186	0.25335	0.25931	0.25633
POR99	0.25496	0.25335	0.25186	0.26528	0.26677	0.23845	0.23845	0.25335	0.25484	0.25633	0.25633	0.25633	0.25633	0.25633	0.25782	0.25931	0.25037	0.25037	0.25037	0.25037	0.25037	0.25186	0.2608	0.25484
SEM144E	0.25648	0.25484	0.25335	0.26379	0.26528	0.23696	0.23696	0.25186	0.25335	0.25484	0.25633	0.25633	0.25633	0.25633	0.25782	0.25633	0.25037	0.25037	0.25037	0.25037	0.25037	0.25186	0.25782	0.25484
N.expDes	0.22925	0.23249	0.231	0.22653	0.22802	0.22802	0.22802	0.23696	0.23845	0.24441	0.24888	0.24888	0.24888	0.24888	0.25037	0.2459	0.24292	0.24292	0.24292	0.24292	0.24292	0.24441	0.2459	0.24441
CHE136	0.22772	0.231	0.22951	0.22802	0.22951	0.22951	0.22951	0.23845	0.23994	0.2459	0.24739	0.24739	0.24739	0.24739	0.24888	0.24739	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.24739	0.24292
KEM114	0.24285	0.24441	0.24292	0.23547	0.23398	0.22355	0.22355	0.24292	0.24441	0.24739	0.24441	0.24441	0.24441	0.24441	0.2459	0.24143	0.25484	0.25484	0.25484	0.25484	0.25484	0.25633	0.25633	0.25633
LAY85	0.2367	0.23696	0.23547	0.22802	0.22951	0.22206	0.22206	0.24134	0.24292	0.23994	0.23994	0.23994	0.23994	0.23994	0.24143	0.23994	0.24888	0.24888	0.24888	0.24888	0.24888	0.25037	0.24441	0.25037
BES40	0.23181	0.23107	0.22957	0.23697	0.23851	0.21116	0.21116	0.22189	0.22343	0.23565	0.23428	0.23428	0.23428	0.23428	0.23582	0.23269	0.23316	0.23316	0.23316	0.23316	0.23316	0.23466	0.22984	0.22535
BIS58	0.23058	0.231	0.22951	0.23696	0.23845	0.2161	0.2161	0.22355	0.22504	0.23696	0.23398	0.23398	0.23398	0.23398	0.23547	0.231	0.23696	0.23696	0.23696	0.23696	0.23696	0.23845	0.231	0.22951
BIS73	0.23216	0.23249	0.231	0.24292	0.24441	0.22057	0.22057	0.22355	0.22504	0.23845	0.23845	0.23845	0.23845	0.23845	0.23994	0.23845	0.23398	0.23398	0.23398	0.23398	0.23398	0.23547	0.23249	0.22355
KAT49	0.23518	0.23547	0.23398	0.24143	0.24292	0.22951	0.22951	0.22653	0.22802	0.23845	0.24441	0.24441	0.24441	0.24441	0.2359	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.2459	0.24292	0.23696
NIP50B	0.23526	0.23547	0.23398	0.23994	0.24143	0.21759	0.21759	0.231	0.23249	0.23696	0.25633	0.25633	0.25633	0.25633	0.25782	0.25633	0.23994	0.23994	0.23994	0.23994	0.23994	0.24143	0.2459	0.2459
GIL70	0.23526	0.23547	0.23398	0.23994	0.24143	0.21759	0.21759	0.231	0.23249	0.23696	0.25633	0.25633	0.25633	0.25633	0.25782	0.25633	0.23994	0.23994	0.23994	0.23994	0.23994	0.24143	0.2459	0.2459
GIL44	0.22426	0.22431	0.22273	0.23111	0.23278	0.20607	0.20607	0.22161	0.22328	0.22768	0.24725	0.24725	0.24725	0.24725	0.24892	0.24728	0.22619	0.22619	0.22619	0.22619	0.22619	0.22767	0.2312	0.2312
ALO171	0.23528	0.23547	0.23398	0.24143	0.24292	0.21461	0.21461	0.231	0.23249	0.23845	0.25484	0.25484	0.25484	0.25484	0.25633	0.25484	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.2459	0.24441
N.expHam	0.23361	0.23547	0.23398	0.23845	0.23944	0.22355	0.22355	0.23398	0.23547	0.25484	0.2459	0.2459	0.2459	0.2459	0.24739	0.24292	0.25037	0.25037	0.25037	0.25037	0.25037	0.25186	0.25037	0.24888
LAY54	0.25658	0.2608	0.25931	0.2608	0.25931	0.25484	0.25484	0.25782	0.25931	0.25931	0.25037	0.25037	0.25037	0.25037	0.25186	0.25037	0.25782	0.25782	0.25782	0.25782	0.25782	0.25653	0.25037	0.26379
P.roscoff	0.29646	0.29779	0.29784	0.2951	0.29155	0.29924	0.29924	0.29331	0.29332	0.29039	0.28731	0.28731	0.28731	0.28731	0.28577	0.28571	0.30401	0.30401	0.30401	0.30401	0.30401	0.30549	0.30502	0.29651
D.simplex	0.28396	0.28316	0.28167	0.25931	0.2608	0.25931	0.25931	0.26975	0.26826	0.26826	0.24739	0.24739	0.24739	0.24739	0.24888	0.25037	0.25484	0.25484	0.25484	0.25484	0.25484	0.25633	0.25633	0.25335
DipspB	0.28245	0.28167	0.28018	0.25782	0.25931	0.25782	0.25782	0.26826	0.26677	0.26677	0.2459	0.2459	0.2459	0.2459	0.24739	0.24888	0.25335	0.25335	0.25335	0.25335	0.25335	0.25484	0.25484	0.25186
POR33	0.25842	0.25931	0.25782	0.25037	0.25186	0.24739	0.24739	0.2623	0.2608	0.2623	0.2459	0.2459	0.2459	0.2459	0.24739	0.24292	0.25484	0.25484	0.25484	0.25484	0.25484	0.25335	0.25633	0.24888
CHD123	0.25842	0.25931	0.25782	0.25037	0.25186	0.24739	0.24739	0.2623	0.2608	0.2623	0.2459	0.2459	0.2459	0.2459	0.24739	0.24292	0.25484	0.25484	0.25484	0.25484	0.25484	0.25335	0.25633	0.24888
POR169	0.25842	0.25931	0.25782	0.25037	0.25186	0.24739	0.24739	0.2623	0.2608	0.2623	0.2459	0.2459	0.2459	0.2459	0.24739	0.24292	0.25484	0.25484	0.25484	0.25484	0.25484	0.25335	0.25633	0.24888
CHD118	0.25842	0.25931	0.25782	0.25037	0.25186	0.24739	0.24739	0.2623	0.2608	0.2623	0.2459	0.2459	0.2459	0.2459	0.24739	0.24292	0.25484	0.25484	0.25484	0.25484	0.25484	0.25335	0.25633	0.24888
TKS128	0.25995	0.2608	0.25931	0.25186	0.25335	0.24888	0.24888	0.26379	0.2623	0.26379	0.24739	0.24739	0.24739	0.24739	0.24888	0.24441	0.25633	0.25633	0.25633	0.25633	0.25633	0.25484	0.25782	0.25037
POR94	0.25689	0.25782	0.25633	0.25186	0.25335	0.24888	0.24888	0.26379	0.2623	0.26379	0.24441	0.24441	0.24441	0.24441	0.2459	0.24441	0.25335	0.25335	0.25335	0.25335	0.25335	0.25816	0.25782	0.24739
CHD121	0.25689	0.25782	0.25633	0.25186	0.25335	0.24888	0.24888	0.26379	0.2623	0.26379	0.24441	0.24441	0.24441	0.24441	0.2459	0.24441	0.25335	0.25335	0.25335	0.25335	0.25335	0.25816	0.25782	0.24739
GIL42	0.26147	0.2623	0.2608	0.25782	0.25931	0.25186	0.25186	0.26677	0.26528	0.26826	0.24739	0.24739	0.24739	0.24739	0.24888	0.24739	0.25782	0.25782	0.25782	0.25782	0.25782	0.25633	0.2623	0.25186
SEM155	0.25996	0.2608	0.25931	0.25186	0.25335	0.24888	0.24888	0.26379	0.2623	0.26379	0.2459	0.2459	0.2459	0.2459	0.24739	0.24292	0.25335	0.25335	0.25335	0.25335	0.25335	0.25816	0.25484	0.24739
POR24	0.25691	0.25782	0.25633	0.25186	0.25335	0.24888	0.24888	0.26379	0.2623	0.26379	0.24143	0.24143	0.24143	0.24143	0.24292	0.24143	0.24888	0.24888	0.24888	0.24888	0.24888	0.24739	0.25335	0.24292
POR26	0.25844	0.25931	0.25782	0.25037	0.25186	0.24739	0.24739	0.2623	0.2608	0.2623	0.24292	0.24292	0.24292	0.24292	0.24441	0.23994	0.25037	0.25037	0.25037	0.25037	0.25037	0.24888	0.25186	0.24441
LAB80	0.2596	0.25931	0.25782	0.25186	0.25335	0.23994	0.23994	0.25037	0.24888	0.26528	0.23249	0.23249	0.23249	0.23249	0.23398	0.22951	0.231	0.231	0.231	0.231	0.231	0.23249	0.24292	0.24292
LAB84	0.2596	0.25931	0.25782	0.25186	0.25335	0.23994	0.23994	0.25037	0.24888	0.26528	0.23249	0.23249	0.23249	0.23249	0.23398	0.22951	0.231	0.231	0.231	0.231	0.231	0.23249	0.24292	0.24292
LAB75	0.2596	0.25931	0.25782	0.25186	0.25335	0.23994	0.23994	0.25037	0.24888	0.26528	0.23249	0.23249	0.23249	0.23249	0.23398	0.22951	0.231	0.231	0.231	0.231	0.231	0.23249	0.24292	0.24292
RAM65	0.2596	0.25931	0.25782	0.25186	0.25335	0.23994	0.23994	0.25037	0.24888	0.26528	0.23249	0.23249	0.23249	0.23249	0.23398	0.22951	0.231	0.231	0.231	0.231	0.231	0.23249	0.24292	0.24292
LAB82	0.2596	0.25931	0.25782	0.25186	0.25335	0.23994	0.23994	0.25037	0.24888	0.26528	0.23249	0.23249	0.23249	0.23249	0.23398	0.22951	0.231	0.231	0.231	0.231	0.231	0.23249	0.24292	0.24292
LAB46	0.2596	0.25931	0.25782	0.25186	0.25335	0.23994	0.23994	0.25037	0.24888	0.26528	0.23249	0.23249	0.23249	0.23249	0.23398	0.22951	0.231	0.231	0.231	0.231	0.231	0.23249	0.24292	0.24292
RAM47A	0.25807	0.25782	0.25633	0.25035	0.25186	0.23845	0.23845	0.24888	0.24739	0.26379	0.23398	0.23398	0.23398	0.23398	0.23547	0.231	0.23249	0.23249	0.23249	0.23249	0.23249	0.23398	0.24441	0.24441
CHE141	0.26914	0.26975	0.26826	0.27124	0.27273	0.24888	0.24888	0.2772	0.27869	0.26826	0.24441	0.24441	0.24441	0.24441	0.2459	0.24441	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.25484	0.24888
CHE143C	0.26914	0.26975	0.26826	0.27124	0.27273	0.24888	0.24888	0.2772	0.27869	0.26826	0.24441	0.24441	0.24441	0.24441	0.2459	0.24441	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.25484	0.24888
SAY134	0.26914	0.26975	0.26826	0.27124	0.27273	0.24888	0.24888	0.2772	0.27869	0.26826	0.24441	0.24441	0.24441	0.24441	0.2459	0.24441	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.25484	0.24888
SAY135	0.26914	0.26975	0.26826	0.27124	0.27273	0.24888	0.24888	0.2772	0.27869	0.26826	0.24441	0.24441	0.24441	0.24441	0.2459	0.24441	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.25484	0.24888
CHE139B	0.26914	0.26975	0.26826	0.27124	0.27273	0.24888	0.24888	0.2772	0.27869	0.26826	0.24441	0.24441	0.24441	0.24441	0.2459	0.24441	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.25484	0.24888

Appendix D (continued)

	CHD122A	CHD120	KU1065	BIS62	BIS63	SEM159A	SEM161B	MER14	MER15	CHE1	MesspC	CHE138C	TKS127A	CHE143A	TKR132	POR34	KEM112	CHD122B	CHD117B	KEM115	KEM111	KEM113	LEN41	CHE140D
CHE137	0.26914	0.26975	0.26826	0.27124	0.27273	0.24888	0.24888	0.2772	0.27869	0.26826	0.24441	0.24441	0.24441	0.24441	0.2459	0.24441	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.25484	0.24888
USA37	0.2749	0.27422	0.27273	0.2623	0.2608	0.27124	0.27124	0.27124	0.26975	0.26528	0.26826	0.26826	0.26826	0.26826	0.26975	0.26528	0.2772	0.2772	0.2772	0.2772	0.2772	0.27571	0.27124	0.26826
MER20	0.36007	0.35917	0.36066	0.36215	0.36215	0.3532	0.3532	0.3532	0.35171	0.36364	0.36811	0.36811	0.36811	0.36811	0.3696	0.36513	0.34575	0.34575	0.34575	0.34575	0.34575	0.34724	0.36066	0.34575
GIL45	0.36007	0.35917	0.36066	0.36215	0.36215	0.3532	0.3532	0.3532	0.35171	0.36364	0.36811	0.36811	0.36811	0.36811	0.3696	0.36513	0.34575	0.34575	0.34575	0.34575	0.34575	0.34724	0.36066	0.34575
GIL57A	0.36007	0.35917	0.36066	0.36215	0.36215	0.3532	0.3532	0.3532	0.35171	0.36364	0.36811	0.36811	0.36811	0.36811	0.3696	0.36513	0.34575	0.34575	0.34575	0.34575	0.34575	0.34724	0.36066	0.34575
GIL89	0.36007	0.35917	0.36066	0.36215	0.36215	0.3532	0.3532	0.3532	0.35171	0.36364	0.36811	0.36811	0.36811	0.36811	0.3696	0.36513	0.34575	0.34575	0.34575	0.34575	0.34575	0.34724	0.36066	0.34575
RAM47B	0.36002	0.35768	0.35917	0.36066	0.36066	0.35171	0.35171	0.35171	0.35022	0.36215	0.36662	0.36662	0.36662	0.36662	0.36811	0.36364	0.34426	0.34426	0.34426	0.34426	0.34426	0.34575	0.35917	0.34426
SEM146	0.35549	0.35469	0.35618	0.35768	0.35768	0.34873	0.34873	0.35469	0.3532	0.36215	0.36513	0.36513	0.36513	0.36513	0.36662	0.36215	0.34426	0.34426	0.34426	0.34426	0.34426	0.34575	0.35917	0.34426
SEM152E	0.35394	0.3532	0.35469	0.3532	0.3532	0.35171	0.35171	0.35917	0.35768	0.37109	0.36811	0.36811	0.36811	0.36811	0.3696	0.36513	0.35022	0.35022	0.35022	0.35022	0.35022	0.35171	0.35768	0.33979
SEM160C	0.35715	0.35469	0.35618	0.35171	0.35171	0.34873	0.34873	0.35768	0.35618	0.36513	0.36811	0.36811	0.36811	0.36811	0.3696	0.36513	0.34724	0.34724	0.34724	0.34724	0.34724	0.34873	0.35469	0.3383
LAB53	0.34926	0.35022	0.35171	0.34277	0.34277	0.34426	0.34426	0.35171	0.35022	0.35917	0.35171	0.35171	0.35171	0.35171	0.3532	0.34873	0.35022	0.35022	0.35022	0.35022	0.35022	0.35171	0.3532	0.33979

Appendix D (continued)

	GIL88	GIL57B	CHE140F	CHE5	LAY86	MER19	MesspD	GIL38	BAT39	GIL56	FUS3	FUS2	NIP66	SEM159D	LAB76	LAB83	LAB81	LAB74	LAB79	LAY55	Rfungi	Ajapon	CHE4	MER21
GIL88	N																							
GIL57B	0.00149	N																						
CHE140F	0.19374	0.19523	N																					
CHE5	0.19225	0.19374	0.00149	N																				
LAY86	0.19523	0.19672	0.00596	0.00745	N																			
MER19	0.1924	0.19389	0.01495	0.01644	0.01493	N																		
MesspD	0.18778	0.18927	0.02086	0.02235	0.01937	0.0284	N																	
GIL38	0.21759	0.2161	0.20566	0.20417	0.20417	0.20294	0.21013	N																
BAT39	0.21759	0.2161	0.20566	0.20417	0.20417	0.20294	0.21013	0	N															
GIL56	0.21759	0.2161	0.20566	0.20417	0.20417	0.20294	0.21013	0	0	N														
FUS3	0.21461	0.21311	0.20268	0.20119	0.20119	0.19995	0.20715	0.00298	0.00298	0.00298	N													
FUS2	0.21461	0.21311	0.20268	0.20119	0.20119	0.19995	0.20715	0.00298	0.00298	0.00298	0	N												
NIP66	0.2161	0.21461	0.20417	0.20268	0.20268	0.20144	0.20864	0.00149	0.00149	0.00149	0.00149	0.00149	N											
SEM159D	0.21461	0.21311	0.20268	0.20119	0.20119	0.19995	0.20715	0.00596	0.00596	0.00596	0.00298	0.00298	0.00447	N										
LAB76	0.20417	0.20566	0.21162	0.21013	0.21311	0.2118	0.21759	0.19076	0.19076	0.19076	0.19374	0.19374	0.19225	0.19374	N									
LAB83	0.20417	0.20566	0.21162	0.21013	0.21311	0.2118	0.21759	0.19076	0.19076	0.19076	0.19374	0.19374	0.19225	0.19374	0	N								
LAB81	0.20417	0.20566	0.21162	0.21013	0.21311	0.2118	0.21759	0.19076	0.19076	0.19076	0.19374	0.19374	0.19225	0.19374	0	0	N							
LAB74	0.20417	0.20566	0.21162	0.21013	0.21311	0.2118	0.21759	0.19076	0.19076	0.19076	0.19374	0.19374	0.19225	0.19374	0	0	0	N						
LAB79	0.20417	0.20566	0.21162	0.21013	0.21311	0.2118	0.21759	0.19076	0.19076	0.19076	0.19374	0.19374	0.19225	0.19374	0	0	0	0	N					
LAY55	0.20417	0.20566	0.21162	0.21013	0.21311	0.2118	0.21759	0.19076	0.19076	0.19076	0.19374	0.19374	0.19225	0.19374	0	0	0	0	0	N				
R.fungi	0.24464	0.24441	0.26014	0.25862	0.26167	0.26174	0.25543	0.2582	0.2582	0.2582	0.25976	0.25976	0.25973	0.25976	0.26001	0.26001	0.26001	0.26001	0.26001	0.26001	N			
A. japon	0.25344	0.25329	0.26568	0.26569	0.26721	0.26283	0.26403	0.25191	0.25191	0.25191	0.24889	0.24889	0.2504	0.24586	0.26419	0.26419	0.26419	0.26419	0.26419	0.26419	0.21163	N		
CHE4	0.24668	0.24821	0.27114	0.2696	0.27267	0.27114	0.27091	0.23703	0.23703	0.23703	0.23703	0.23703	0.2355	0.23703	0.24942	0.24942	0.24942	0.24942	0.24942	0.24942	0.22864	0.22226	N	
MER21	0.24739	0.24888	0.27273	0.27124	0.27422	0.27146	0.27124	0.23547	0.23547	0.23547	0.23547	0.23547	0.23398	0.23547	0.25037	0.25037	0.25037	0.25037	0.25037	0.25037	0.22843	0.22338	0	N
MER91	0.24739	0.24888	0.27273	0.27124	0.27422	0.27146	0.27124	0.23547	0.23547	0.23547	0.23547	0.23547	0.23398	0.23547	0.25037	0.25037	0.25037	0.25037	0.25037	0.25037	0.22843	0.22338	0	0
CHE6	0.24739	0.24888	0.27273	0.27124	0.27422	0.27146	0.27124	0.23547	0.23547	0.23547	0.23547	0.23547	0.23398	0.23547	0.25037	0.25037	0.25037	0.25037	0.25037	0.25037	0.22843	0.22338	0	0
POR27	0.24739	0.24888	0.27273	0.27124	0.27422	0.27146	0.27124	0.23547	0.23547	0.23547	0.23547	0.23547	0.23398	0.23547	0.25037	0.25037	0.25037	0.25037	0.25037	0.25037	0.22843	0.22338	0	0
POR100	0.24739	0.24888	0.27273	0.27124	0.27422	0.27146	0.27124	0.23547	0.23547	0.23547	0.23547	0.23547	0.23398	0.23547	0.25037	0.25037	0.25037	0.25037	0.25037	0.25037	0.22843	0.22338	0	0
MER95	0.24739	0.2459	0.27273	0.27124	0.27422	0.27146	0.27124	0.23249	0.23249	0.23249	0.23249	0.23249	0.231	0.23249	0.25037	0.25037	0.25037	0.25037	0.25037	0.25037	0.22813	0.22307	0.00155	0.00298
CHE2	0.24143	0.23994	0.25782	0.25633	0.25931	0.25354	0.2623	0.24143	0.24143	0.24143	0.24143	0.24143	0.23994	0.23845	0.24292	0.24292	0.24292	0.24292	0.24292	0.24292	0.2415	0.22911	0.21594	0.21461
POR25	0.24292	0.24143	0.25931	0.25782	0.2608	0.25503	0.26379	0.24292	0.24292	0.24292	0.24292	0.24292	0.24143	0.23994	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.24184	0.22938	0.2161	0.21311
MER64	0.24292	0.24143	0.25931	0.25782	0.2608	0.25503	0.26379	0.24292	0.24292	0.24292	0.24292	0.24292	0.24143	0.23994	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.23999	0.22761	0.21441	0.21311
LAY51	0.23985	0.23832	0.25628	0.25473	0.25788	0.25318	0.26233	0.24227	0.24227	0.24227	0.24228	0.24228	0.24073	0.23918	0.2422	0.2422	0.2422	0.2422	0.2422	0.2422	0.23862	0.22708	0.21175	0.21174
LAY52	0.23994	0.23845	0.25633	0.25484	0.25782	0.25205	0.2608	0.23845	0.23845	0.23845	0.23845	0.23845	0.23696	0.23547	0.24143	0.24143	0.24143	0.24143	0.24143	0.24143	0.24302	0.22606	0.21289	0.21162
GIL48B	0.24015	0.23866	0.25659	0.2551	0.25808	0.2523	0.26106	0.23867	0.23867	0.23867	0.23868	0.23868	0.23718	0.2357	0.24166	0.24166	0.24166	0.24166	0.24166	0.24166	0.24327	0.22632	0.21308	0.21182
POR167	0.24143	0.23994	0.25484	0.25335	0.25633	0.25055	0.25931	0.24143	0.24143	0.24143	0.24143	0.24143	0.23994	0.23845	0.2459	0.2459	0.2459	0.2459	0.2459	0.2459	0.23998	0.23666	0.20828	0.20715
POR99	0.24292	0.24143	0.25633	0.25484	0.25782	0.25204	0.2608	0.24292	0.24292	0.24292	0.24292	0.24292	0.24143	0.23994	0.24739	0.24739	0.24739	0.24739	0.24739	0.24739	0.24032	0.23694	0.20846	0.20566
SEM144E	0.24292	0.24143	0.25335	0.25186	0.25484	0.24906	0.25782	0.24292	0.24292	0.24292	0.24292	0.24292	0.24143	0.23994	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.23841	0.23509	0.20983	0.20864
N.expDes	0.24888	0.24888	0.25633	0.25484	0.25633	0.25059	0.2608	0.24888	0.24888	0.24888	0.25186	0.25186	0.25037	0.25484	0.24441	0.24441	0.24441	0.24441	0.24441	0.24441	0.22931	0.24107	0.20959	0.20715
CHE136	0.25037	0.25037	0.25782	0.25633	0.25782	0.25208	0.2623	0.25037	0.25037	0.25037	0.25335	0.25335	0.25186	0.25633	0.2459	0.2459	0.2459	0.2459	0.2459	0.2459	0.22957	0.24136	0.20971	0.20566
KEM114	0.25484	0.25484	0.25484	0.25633	0.25484	0.24907	0.25633	0.25335	0.25335	0.25335	0.25633	0.25633	0.25484	0.25931	0.25931	0.25931	0.25931	0.25931	0.25931	0.25931	0.22183	0.23213	0.21095	0.20864
LAY85	0.25335	0.25335	0.2459	0.24441	0.24739	0.24457	0.25633	0.25782	0.25782	0.25782	0.25782	0.25782	0.25633	0.2608	0.24888	0.24888	0.24888	0.24888	0.24888	0.24888	0.222	0.23688	0.2095	0.20715
BES40	0.23593	0.23568	0.24933	0.2484	0.24993	0.24402	0.24841	0.24155	0.24155	0.24155	0.24463	0.24463	0.2431	0.24769	0.24344	0.24344	0.24344	0.24344	0.24344	0.24344	0.20776	0.22357	0.21121	0.20815
BIS58	0.23994	0.23845	0.24888	0.24739	0.24888	0.24312	0.25037	0.24143	0.24143	0.24143	0.24441	0.24441	0.24292	0.24739	0.24292	0.24292	0.24292	0.24292	0.24292	0.24292	0.20811	0.23366	0.21419	0.21162
BIS73	0.24143	0.24143	0.25186	0.25037	0.25186	0.2461	0.25335	0.24739	0.24739	0.24739	0.25037	0.25037	0.24888	0.25335	0.24888	0.24888	0.24888	0.24888	0.24888	0.24888	0.21302	0.23682	0.2158	0.21162
KAT49	0.25186	0.25186	0.25186	0.25037	0.25186	0.2461	0.25186	0.2459	0.2459	0.2459	0.24888	0.24888	0.24739	0.25186	0.24888	0.24888	0.24888	0.24888	0.24888	0.24888	0.21895	0.23832	0.21875	0.21461
NIP50B	0.25186	0.25186	0.24739	0.2459	0.24739	0.24162	0.25186	0.23547	0.23547	0.23547	0.23547	0.23547	0.23398	0.23845	0.25186	0.25186	0.25186	0.25186	0.25186	0.25186	0.22641	0.2444	0.21878	0.21461
GIL70	0.25186	0.25186	0.24739	0.2459	0.24739	0.24162	0.25186	0.23547	0.23547	0.23547	0.23547	0.23547	0.23398	0.23845	0.25186	0.25186	0.25186	0.25186	0.25186	0.25186	0.22641	0.2444	0.21878	0.21461
GIL44	0.23637	0.2363	0.22984	0.22972	0.22985	0.22479	0.23491	0.22601	0.22601	0.22601	0.22603	0.22603	0.22437	0.22937	0.24103	0.24103	0.24103	0.24103	0.24103	0.24103	0.22422	0.23243	0.21629	0.21148
ALO171	0.24739	0.24739	0.2459	0.24441	0.2459	0.24013	0.25037	0.23547	0.23547	0.23547	0.23547	0.23547	0.23398	0.23845	0.25037	0.25037	0.25037	0.25037	0.25037	0.25037	0.21275	0.23836	0.21276	0.20864
N.expHam	0.24143	0.24143	0.26379	0.2623	0.26379	0.25804	0.26826	0.24441	0.24441	0.24441	0.24739	0.24739	0.2459	0.25037	0.24739	0.24739	0.24739	0.24739	0.24739	0.24739	0.21102	0.23816	0.20346	0.20119

Appendix D (continued)

	GIL88	GIL57B	CHE140F	CHE5	LAY86	MER19	MesspD	GIL38	BAT39	GIL56	FUS3	FUS2	NIP66	SEM159D	LAB76	LAB83	LAB81	LAB74	LAB79	LAY55	Rfungi	Ajapon	CHE4	MER21
LAY54	0.25931	0.25782	0.24441	0.2459	0.24441	0.24158	0.24739	0.24441	0.24441	0.24441	0.24143	0.24143	0.24292	0.23845	0.25782	0.25782	0.25782	0.25782	0.25782	0.25782	0.21541	0.23358	0.24602	0.24441
P.roscoff	0.3038	0.30363	0.30396	0.30244	0.30093	0.29936	0.30529	0.29271	0.29271	0.29271	0.28969	0.28969	0.2912	0.28665	0.29308	0.29308	0.29308	0.29308	0.29308	0.29308	0.31054	0.27381	0.30642	0.30595
D.simplex	0.26379	0.2623	0.26379	0.26528	0.26379	0.25959	0.26677	0.23994	0.23994	0.23994	0.23994	0.23994	0.24143	0.23994	0.24739	0.24739	0.24739	0.24739	0.24739	0.24739	0.23703	0.2611	0.2108	0.20715
DipspB	0.2623	0.2608	0.2623	0.26379	0.2623	0.25809	0.26528	0.24143	0.24143	0.24143	0.24143	0.24143	0.24292	0.24143	0.24888	0.24888	0.24888	0.24888	0.24888	0.24888	0.23856	0.26263	0.20929	0.20566
POR33	0.23994	0.24143	0.24292	0.24143	0.24292	0.23696	0.24441	0.24739	0.24739	0.24739	0.24441	0.24441	0.2459	0.244411	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.23263	0.24957	0.2221	0.22057
CHD123	0.23994	0.24143	0.24292	0.24143	0.24292	0.23696	0.24441	0.24739	0.24739	0.24739	0.24441	0.24441	0.2459	0.244411	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.23263	0.24957	0.2221	0.22057
POR169	0.23994	0.24143	0.24292	0.24143	0.24292	0.23696	0.24441	0.24739	0.24739	0.24739	0.24441	0.24441	0.2459	0.244411	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.23263	0.24957	0.2221	0.22057
CHD118	0.23994	0.24143	0.24292	0.24143	0.24292	0.23696	0.24441	0.24739	0.24739	0.24739	0.24441	0.24441	0.2459	0.244411	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.23263	0.24957	0.2221	0.22057
TKS128	0.24143	0.24292	0.24441	0.24292	0.24441	0.23845	0.2459	0.24888	0.24888	0.24888	0.2459	0.2459	0.24739	0.2459	0.22802	0.22802	0.22802	0.22802	0.22802	0.22802	0.23414	0.24748	0.22363	0.22206
POR94	0.24143	0.24292	0.24441	0.24292	0.24441	0.23845	0.2459	0.24888	0.24888	0.24888	0.2459	0.2459	0.24739	0.2459	0.22802	0.22802	0.22802	0.22802	0.22802	0.22802	0.23299	0.24627	0.22229	0.21908
CHD121	0.24143	0.24292	0.24441	0.24292	0.24441	0.23845	0.2459	0.24888	0.24888	0.24888	0.2459	0.2459	0.24739	0.2459	0.22802	0.22802	0.22802	0.22802	0.22802	0.22802	0.23299	0.24627	0.22229	0.21908
GIL42	0.2459	0.24739	0.2459	0.24441	0.2459	0.23994	0.24739	0.25335	0.25335	0.25335	0.25037	0.25037	0.25186	0.25037	0.22951	0.22951	0.22951	0.22951	0.22951	0.22951	0.23451	0.2508	0.22369	0.22206
SEM155	0.23845	0.23994	0.24143	0.23994	0.24143	0.23547	0.24292	0.24888	0.24888	0.24888	0.2459	0.2459	0.24739	0.2459	0.22504	0.22504	0.22504	0.22504	0.22504	0.22504	0.2326	0.24441	0.22212	0.22057
POR24	0.23845	0.23994	0.2459	0.24441	0.2459	0.23994	0.24739	0.24888	0.24888	0.24888	0.2459	0.2459	0.24739	0.2459	0.22802	0.22802	0.22802	0.22802	0.22802	0.22802	0.23601	0.24624	0.22387	0.22057
POR26	0.23696	0.23845	0.24441	0.24292	0.24441	0.23845	0.2459	0.24739	0.24739	0.24739	0.24441	0.24441	0.2459	0.24441	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.23563	0.24592	0.22366	0.22206
LAB80	0.25186	0.25037	0.24888	0.24739	0.24888	0.2459	0.25484	0.25335	0.25335	0.25335	0.25335	0.25335	0.25484	0.25037	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.22925	0.26089	0.22946	0.22653
LAB84	0.25186	0.25037	0.24888	0.24739	0.24888	0.2459	0.25484	0.25335	0.25335	0.25335	0.25335	0.25335	0.25484	0.25037	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.22925	0.26089	0.22946	0.22653
LAB75	0.25186	0.25037	0.24888	0.24739	0.24888	0.2459	0.25484	0.25335	0.25335	0.25335	0.25335	0.25335	0.25484	0.25037	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.22925	0.26089	0.22946	0.22653
RAM65	0.25186	0.25037	0.24888	0.24739	0.24888	0.2459	0.25484	0.25335	0.25335	0.25335	0.25335	0.25335	0.25484	0.25037	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.22925	0.26089	0.22946	0.22653
LAB82	0.25186	0.25037	0.24888	0.24739	0.24888	0.2459	0.25484	0.25335	0.25335	0.25335	0.25335	0.25335	0.25484	0.25037	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.22925	0.26089	0.22946	0.22653
LAB46	0.25186	0.25037	0.24888	0.24739	0.24888	0.2459	0.25484	0.25335	0.25335	0.25335	0.25335	0.25335	0.25484	0.25037	0.23696	0.23696	0.23696	0.23696	0.23696	0.23696	0.22925	0.26089	0.22946	0.22653
RAM47A	0.25335	0.25186	0.25037	0.24888	0.25037	0.24739	0.25633	0.25186	0.25186	0.25186	0.25186	0.25186	0.25335	0.24888	0.23845	0.23845	0.23845	0.23845	0.23845	0.23845	0.22774	0.26243	0.22945	0.22653
CHE141	0.22057	0.22206	0.23994	0.23845	0.23994	0.23547	0.23994	0.23398	0.23398	0.23398	0.231	0.231	0.23249	0.231	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22828	0.24291	0.20952	0.20566
CHE143C	0.22057	0.22206	0.23994	0.23845	0.23994	0.23547	0.23994	0.23398	0.23398	0.23398	0.231	0.231	0.23249	0.231	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22828	0.24291	0.20952	0.20566
SAY134	0.22057	0.22206	0.23994	0.23845	0.23994	0.23547	0.23994	0.23398	0.23398	0.23398	0.231	0.231	0.23249	0.231	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22828	0.24291	0.20952	0.20566
SAY135	0.22057	0.22206	0.23994	0.23845	0.23994	0.23547	0.23994	0.23398	0.23398	0.23398	0.231	0.231	0.23249	0.231	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22828	0.24291	0.20952	0.20566
CHE139B	0.22057	0.22206	0.23994	0.23845	0.23994	0.23547	0.23994	0.23398	0.23398	0.23398	0.231	0.231	0.23249	0.231	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22828	0.24291	0.20952	0.20566
CHE137	0.22057	0.22206	0.23994	0.23845	0.23994	0.23547	0.23994	0.23398	0.23398	0.23398	0.231	0.231	0.23249	0.231	0.22653	0.22653	0.22653	0.22653	0.22653	0.22653	0.22828	0.24291	0.20952	0.20566
USA37	0.2772	0.2772	0.25782	0.25931	0.25782	0.25633	0.25186	0.2608	0.2608	0.2608	0.2608	0.2608	0.25931	0.2608	0.28614	0.28614	0.28614	0.28614	0.28614	0.28614	0.23702	0.24593	0.24381	0.23994
MER20	0.35768	0.35917	0.35618	0.35678	0.35768	0.34896	0.35917	0.3532	0.3532	0.3532	0.35022	0.35022	0.35171	0.34724	0.35022	0.35022	0.35022	0.35022	0.35022	0.35022	0.35716	0.36745	0.37021	0.36662
GIL45	0.35768	0.35917	0.35618	0.35678	0.35768	0.34896	0.35917	0.3532	0.3532	0.3532	0.35022	0.35022	0.35171	0.34724	0.35022	0.35022	0.35022	0.35022	0.35022	0.35022	0.35716	0.36745	0.37021	0.36662
GIL57A	0.35768	0.35917	0.35618	0.35678	0.35768	0.34896	0.35917	0.3532	0.3532	0.3532	0.35022	0.35022	0.35171	0.34724	0.35022	0.35022	0.35022	0.35022	0.35022	0.35022	0.35716	0.36745	0.37021	0.36662
GIL89	0.35768	0.35917	0.35618	0.35678	0.35768	0.34896	0.35917	0.3532	0.3532	0.3532	0.35022	0.35022	0.35171	0.34724	0.35022	0.35022	0.35022	0.35022	0.35022	0.35022	0.35716	0.36745	0.37021	0.36662
RAM47B	0.35618	0.35768	0.35768	0.35917	0.35917	0.35045	0.36066	0.35171	0.35171	0.35171	0.34873	0.34873	0.35022	0.34575	0.34873	0.34873	0.34873	0.34873	0.34873	0.34873	0.35563	0.36592	0.36868	0.36513
SEM146	0.35618	0.35768	0.35618	0.35768	0.35618	0.34747	0.35768	0.35171	0.35171	0.35171	0.34873	0.34873	0.35022	0.34575	0.34575	0.34575	0.34575	0.34575	0.34575	0.34575	0.35716	0.36741	0.36715	0.36364
SEM152E	0.35917	0.36066	0.35917	0.36066	0.35917	0.35343	0.35917	0.3532	0.3532	0.3532	0.3532	0.3532	0.35171	0.35022	0.34724	0.34724	0.34724	0.34724	0.34724	0.34724	0.36774	0.36293	0.37172	0.36811
SEM160C	0.35618	0.35768	0.36066	0.36215	0.36066	0.35492	0.35768	0.3532	0.3532	0.3532	0.3532	0.3532	0.35171	0.35022	0.34277	0.34277	0.34277	0.34277	0.34277	0.34277	0.36641	0.3614	0.36865	0.36513
LAB53	0.34873	0.35022	0.35469	0.35618	0.35618	0.34895	0.3532	0.34873	0.34873	0.34873	0.35171	0.35171	0.35022	0.35469	0.34724	0.34724	0.34724	0.34724	0.34724	0.34724	0.36458	0.38096	0.3744	0.37109

Appendix D (continued)

	MER91	CHE6	POR27	POR100	MER95	CHE2	POR25	MER64	LAY51	LAY52	GIL48B	POR167	POR99	SEM144E	NexpDes	CHE136	KEM114	LAY85	BES40	BIS58	BIS73	KAT49	NIP50B	GIL70
MER91	N																							
CHE6	0	N																						
POR27	0	0	N																					
POR100	0	0	0	N																				
MER95	0.00298	0.00298	0.00298	0.00298	N																			
CHE2	0.21461	0.21461	0.21461	0.21461	0.21162	N																		
POR25	0.21311	0.21311	0.21311	0.21311	0.21311	0.00149	N																	
MER64	0.21311	0.21311	0.21311	0.21311	0.21013	0.00149	0.00298	N																
LAY51	0.21174	0.21174	0.21174	0.21174	0.21003	0.00458	0.00457	0.00615	N															
LAY52	0.21162	0.21162	0.21162	0.21162	0.20864	0.00447	0.00596	0.00596	0	N														
GIL48B	0.21182	0.21182	0.21182	0.21182	0.20884	0.00448	0.00597	0.00597	0	0	N													
POR167	0.20715	0.20715	0.20715	0.20715	0.20417	0.02086	0.02235	0.02235	0.02612	0.02534	0.02537	N												
POR99	0.20566	0.20566	0.20566	0.20566	0.20566	0.02235	0.02086	0.02385	0.0261	0.02683	0.02686	0.00149	N											
SEM144E	0.20864	0.20864	0.20864	0.20864	0.20566	0.02086	0.02235	0.02235	0.02612	0.02534	0.02537	0.00298	0.00447	N										
N.expDes	0.20715	0.20715	0.20715	0.20715	0.20566	0.23398	0.23547	0.23547	0.23919	0.23547	0.23564	0.22951	0.231	0.22802	N									
CHE136	0.20566	0.20566	0.20566	0.20566	0.20715	0.23547	0.23398	0.23696	0.2393	0.23696	0.23713	0.231	0.22951	0.22951	0.00149	N								
KEM114	0.20864	0.20864	0.20864	0.20864	0.20715	0.24143	0.24292	0.24292	0.24672	0.24292	0.2431	0.23696	0.23845	0.23547	0.0462	0.04769	N							
LAY85	0.20715	0.20715	0.20715	0.20715	0.20864	0.23994	0.23845	0.24143	0.2422	0.24143	0.24162	0.23994	0.23845	0.23845	0.06706	0.06557	0.06259	N						
BES40	0.20815	0.20815	0.20815	0.20815	0.2079	0.23391	0.23408	0.23544	0.23675	0.23543	0.23561	0.23386	0.23405	0.23228	0.06566	0.06571	0.06122	0.0673	N					
BIS58	0.21162	0.21162	0.21162	0.21162	0.20864	0.231	0.23249	0.23249	0.2347	0.23249	0.23265	0.22951	0.231	0.22802	0.06408	0.06557	0.05812	0.07452	0.01223	N				
BIS73	0.21162	0.21162	0.21162	0.21162	0.21311	0.23398	0.23249	0.23547	0.23622	0.23547	0.23563	0.23249	0.231	0.231	0.06259	0.0611	0.05663	0.06855	0.01531	0.01639	N			
KAT49	0.21461	0.21461	0.21461	0.21461	0.2161	0.23696	0.23547	0.23845	0.2392	0.23845	0.23863	0.23249	0.231	0.231	0.6706	0.06557	0.06557	0.07154	0.03365	0.03577	0.02832	N		
NIP50B	0.21461	0.21461	0.21461	0.21461	0.2161	0.23845	0.23696	0.23994	0.24083	0.23994	0.2401	0.23249	0.231	0.231	0.07303	0.07154	0.06557	0.07154	0.0656	0.06855	0.06706	0.06557	N	
GIL70	0.21461	0.21461	0.21461	0.21461	0.2161	0.23845	0.23696	0.23994	0.24083	0.23994	0.2401	0.23249	0.231	0.231	0.07303	0.07154	0.06557	0.07154	0.0656	0.06855	0.06706	0.06557	0	N
GIL44	0.21148	0.21148	0.21148	0.21148	0.21315	0.23276	0.23108	0.23994	0.23542	0.23441	0.2346	0.22603	0.22434	0.22427	0.06984	0.06809	0.06692	0.06691	0.05862	0.06182	0.06017	0.06378	0	0
ALO171	0.20864	0.20864	0.20864	0.20864	0.21013	0.23398	0.23249	0.23547	0.23631	0.23547	0.23563	0.22802	0.22653	0.22653	0.07154	0.07004	0.05961	0.06557	0.05957	0.06259	0.0611	0.0611	0.0149	0.0149
N.expHam	0.20119	0.20119	0.20119	0.20119	0.1997	0.24143	0.24292	0.24292	0.24551	0.24292	0.24309	0.23845	0.23994	0.23547	0.08346	0.08495	0.09389	0.0924	0.07482	0.08048	0.08644	0.08644	0.08793	0.08793
LAY54	0.24441	0.24441	0.24441	0.24441	0.24143	0.23547	0.23696	0.23398	0.23588	0.23845	0.2387	0.23994	0.24143	0.23994	0.22504	0.22653	0.22802	0.22504	0.22637	0.22653	0.22802	0.22653	0.231	0.231
P.roscoff	0.30595	0.30595	0.30595	0.30595	0.30563	0.28277	0.28298	0.28124	0.28399	0.28128	0.28145	0.28122	0.28141	0.28119	0.2992	0.29937	0.30394	0.31473	0.29845	0.29465	0.30255	0.30245	0.30227	0.30227
D.simplex	0.20715	0.20715	0.20715	0.20715	0.20715	0.20268	0.20119	0.20119	0.19904	0.1997	0.19981	0.21013	0.20864	0.21162	0.24292	0.24143	0.2459	0.25037	0.23825	0.24143	0.23845	0.24739	0.25484	0.25484
DipspB	0.20566	0.20566	0.20566	0.20566	0.20566	0.20119	0.1997	0.1997	0.19752	0.19821	0.19832	0.20864	0.20715	0.21013	0.24143	0.23994	0.24441	0.24888	0.23674	0.23994	0.23696	0.2459	0.25335	0.25335
POR33	0.22057	0.22057	0.22057	0.22057	0.22057	0.19672	0.19821	0.19821	0.19486	0.19374	0.19383	0.19523	0.19672	0.19523	0.20566	0.20715	0.22355	0.22504	0.20659	0.21013	0.21461	0.21908	0.21013	0.21013
CHD123	0.22057	0.22057	0.22057	0.22057	0.22057	0.19672	0.19821	0.19821	0.19486	0.19374	0.19383	0.19523	0.19672	0.19523	0.20566	0.20715	0.22355	0.22504	0.20659	0.21013	0.21461	0.21908	0.21013	0.21013
POR169	0.22057	0.22057	0.22057	0.22057	0.22057	0.19672	0.19821	0.19821	0.19486	0.19374	0.19383	0.19523	0.19672	0.19523	0.20566	0.20715	0.22355	0.22504	0.20659	0.21013	0.21461	0.21908	0.21013	0.21013
CHD118	0.22057	0.22057	0.22057	0.22057	0.22057	0.19672	0.19821	0.19821	0.19486	0.19374	0.19383	0.19523	0.19672	0.19523	0.20566	0.20715	0.22355	0.22504	0.20659	0.21013	0.21461	0.21908	0.21013	0.21013
TKS128	0.22206	0.22206	0.22206	0.22206	0.22206	0.19821	0.1997	0.1997	0.19639	0.19523	0.19532	0.19672	0.19821	0.19672	0.20715	0.20864	0.22504	0.22653	0.20811	0.21162	0.2161	0.22057	0.21162	0.21162
POR94	0.21908	0.21908	0.21908	0.21908	0.22206	0.19821	0.19672	0.1997	0.19499	0.19523	0.19532	0.19672	0.19523	0.19672	0.20715	0.20566	0.22504	0.22355	0.20681	0.21162	0.21311	0.21759	0.20864	0.20864
CHD121	0.21908	0.21908	0.21908	0.21908	0.22206	0.19821	0.19672	0.1997	0.19499	0.19523	0.19532	0.19672	0.19523	0.19672	0.20715	0.20566	0.22504	0.22355	0.20681	0.21162	0.21311	0.21759	0.20864	0.20864
GIL42	0.22206	0.22206	0.22206	0.22206	0.22504	0.20268	0.20119	0.20417	0.19802	0.1997	0.19979	0.20119	0.1997	0.20119	0.21162	0.21013	0.22802	0.22653	0.21139	0.2161	0.21759	0.22206	0.21162	0.21162
SEM155	0.22057	0.22057	0.22057	0.22057	0.22057	0.19821	0.1997	0.1997	0.19641	0.19523	0.19532	0.19672	0.19821	0.19672	0.20417	0.20566	0.22206	0.22355	0.20503	0.20864	0.21311	0.21759	0.20864	0.20864
POR24	0.22057	0.22057	0.22057	0.22057	0.22355	0.20119	0.1997	0.20268	0.19812	0.19821	0.1983	0.1997	0.19821	0.1997	0.20715	0.20566	0.22504	0.22355	0.2068	0.21162	0.21311	0.21759	0.20864	0.20864
POR26	0.22206	0.22206	0.22206	0.22206	0.22206	0.1997	0.20119	0.20119	0.19797	0.19672	0.19681	0.19821	0.1997	0.19821	0.20566	0.20715	0.22355	0.22504	0.20657	0.21013	0.21461	0.21908	0.21013	0.21013
LAB80	0.22653	0.22653	0.22653	0.22653	0.22355	0.20119	0.20268	0.20268	0.19918	0.19821	0.19834	0.19672	0.19821	0.1997	0.22057	0.22206	0.21908	0.22504	0.20787	0.20268	0.20864	0.2161	0.21461	0.21461
LAB84	0.22653	0.22653	0.22653	0.22653	0.22355	0.20119	0.20268	0.20268	0.19918	0.19821	0.19834	0.19672	0.19821	0.1997	0.22057	0.22206	0.21908	0.22504	0.20787	0.20268	0.20864	0.2161	0.21461	0.21461
LAB75	0.22653	0.22653	0.22653	0.22653	0.22355	0.20119	0.20268	0.20268	0.19918	0.19821	0.19834	0.19672	0.19821	0.1997	0.22057	0.22206	0.21908	0.22504	0.20787	0.20268	0.20864	0.2161	0.21461	0.21461
RAM65	0.22653	0.22653	0.22653	0.22653	0.22355	0.20119	0.20268	0.20268	0.19918	0.19821	0.19834	0.19672	0.19821	0.1997	0.22057	0.22206	0.21908	0.22504	0.20787	0.20268	0.20864	0.2161	0.21461	0.21461
LAB82	0.22653	0.22653	0.22653	0.22653	0.22355	0.20119	0.20268	0.20268	0.19918	0.19821	0.19834	0.19672	0.19821	0.1997	0.22057	0.22206	0.21908	0.22504	0.20787	0.20268	0.20864	0.2161	0.21461	0.21461
LAB46	0.22653	0.22653	0.22653	0.22653	0.22355	0.20119	0.20268	0.20268	0.19918	0.19821	0.19834	0.19672	0.19821	0.1997	0.22057	0.22206	0.21908	0.22504	0.20787	0.20268	0.20864	0.2161	0.21461	0.21461
RAM47A	0.22653	0.22653	0.22653	0.22653	0.22355	0.1997	0.20119	0.20119	0.19765	0.19672	0.19685	0.19523	0.19672	0.19821	0.22206	0.22355	0.22057	0.22653	0.20941	0.20417	0.21013	0.21759	0.2161	0.2161
CHE141	0.20566	0.20566	0.20566	0.20566	0.20864	0.19523	0.19374	0.19523	0.19788	0.19821	0.1983	0.19672	0.19523	0.19821	0.21908	0.21759	0.23398	0.23547	0.219	0.21908	0.22355	0.22951	0.21908	0.21908
CHE143C	0.20566	0.20566	0.20566	0.20566	0.20864	0.19523	0.19374	0.19523	0.19788	0.19821	0.1983	0.19672	0.19523	0.19821	0.21908	0.21759	0.23398	0.23547	0.219	0.21908	0.22355	0.22951	0.21908	0.21908

Appendix D (continued)

		CHE6	POR27	POR100	MER95	CHE2	POR25	MER64	LAY51	LAY52	GIL48B	POR167	POR99	SEM144E	NexpDes	CHE136	KEM114	LAY85	BES40	BIS58	BIS73	KAT49	NIP50B	GIL70
SAY134	0.20566	0.20566	0.20566	0.20566	0.20864	0.19523	0.19374	0.19523	0.19788	0.19821	0.1983	0.19672	0.19523	0.19821	0.21908	0.21759	0.23398	0.23547	0.219	0.21908	0.22355	0.22951	0.21908	0.21908
SAY135	0.20566	0.20566	0.20566	0.20566	0.20864	0.19523	0.19374	0.19523	0.19788	0.19821	0.1983	0.19672	0.19523	0.19821	0.21908	0.21759	0.23398	0.23547	0.219	0.21908	0.22355	0.22951	0.21908	0.21908
CHE139B	0.20566	0.20566	0.20566	0.20566	0.20864	0.19523	0.19374	0.19523	0.19788	0.19821	0.1983	0.19672	0.19523	0.19821	0.21908	0.21759	0.23398	0.23547	0.219	0.21908	0.22355	0.22951	0.21908	0.21908
CHE137	0.20566	0.20566	0.20566	0.20566	0.20864	0.19523	0.19374	0.19523	0.19788	0.19821	0.1983	0.19672	0.19523	0.19821	0.21908	0.21759	0.23398	0.23547	0.219	0.21908	0.22355	0.22951	0.21908	0.21908
USA37	0.23994	0.23994	0.23994	0.23994	0.23845	0.27124	0.27273	0.27273	0.27351	0.26975	0.27001	0.26528	0.26677	0.26826	0.25186	0.25335	0.24292	0.2608	0.24485	0.25186	0.25484	0.25186	0.25484	0.25484
MER20	0.36662	0.36662	0.36662	0.36662	0.36662	0.3532	0.35469	0.3532	0.35786	0.3532	0.35352	0.3532	0.35469	0.35171	0.36662	0.36811	0.37407	0.37109	0.36836	0.3696	0.37109	0.37258	0.37258	0.37258
GIL45	0.36662	0.36662	0.36662	0.36662	0.36662	0.3532	0.35469	0.3532	0.35786	0.3532	0.35352	0.3532	0.35469	0.35171	0.36662	0.36811	0.37407	0.37109	0.36836	0.3696	0.37109	0.37258	0.37258	0.37258
GIL57A	0.36662	0.36662	0.36662	0.36662	0.36662	0.3532	0.35469	0.3532	0.35786	0.3532	0.35352	0.3532	0.35469	0.35171	0.36662	0.36811	0.37407	0.37109	0.36836	0.3696	0.37109	0.37258	0.37258	0.37258
GIL89	0.36662	0.36662	0.36662	0.36662	0.36662	0.3532	0.35469	0.3532	0.35786	0.3532	0.35352	0.3532	0.35469	0.35171	0.36662	0.36811	0.37407	0.37109	0.36836	0.3696	0.37109	0.37258	0.37258	0.37258
RAM47B	0.36513	0.36513	0.36513	0.36513	0.36513	0.35171	0.3532	0.35171	0.35633	0.35171	0.35202	0.35171	0.3532	0.35022	0.36513	0.36662	0.37258	0.3696	0.36683	0.36811	0.3696	0.37109	0.37109	0.37109
SEM146	0.36364	0.36364	0.36364	0.36364	0.36364	0.34724	0.34873	0.34724	0.35168	0.34724	0.34755	0.34724	0.34873	0.34575	0.36364	0.36513	0.37109	0.36811	0.36835	0.3696	0.37109	0.37258	0.37258	0.37258
SEM152E	0.36811	0.36811	0.36811	0.36811	0.36811	0.35768	0.35917	0.35768	0.36401	0.35768	0.35795	0.35768	0.35917	0.35618	0.36513	0.36662	0.3696	0.3696	0.36075	0.36513	0.36513	0.36811	0.35768	0.35768
SEM160C	0.36513	0.36513	0.36513	0.36513	0.36513	0.35917	0.36066	0.35917	0.36555	0.35917	0.35944	0.35917	0.36066	0.35768	0.36662	0.36811	0.37109	0.37109	0.35792	0.36662	0.36513	0.3696	0.36215	0.36215
LAB53	0.37109	0.37109	0.37109	0.37109	0.37109	0.34426	0.34575	0.34575	0.34674	0.34426	0.34446	0.33979	0.34128	0.3383	0.34873	0.35022	0.35171	0.35469	0.35436	0.35022	0.35171	0.34873	0.3532	0.3532

Appendix D (continued)

	GIL44	ALO171	NexpHam	LAY54	P.roscoff	Dipsimplex	DipspB	POR33	CHD123	POR169	CHD118	TKS128	POR94	CHD121	GIL42	SEM155	POR24	POR26	LAB80	LAB84	LAB75	RAM65	LAB82	LAB46
GIL44	N																							
ALO171	0.01498	N																						
N.expHam	0.08428	0.08197	N																					
LAY54	0.21931	0.22802	0.23547	N																				
P.roscoff	0.29416	0.30077	0.30692	0.2821	N																			
D.simplex	0.24757	0.25186	0.23696	0.23398	0.29364	N																		
DipspB	0.24592	0.25037	0.23547	0.23249	0.29213	0.00149	N																	
POR33	0.20649	0.21013	0.21013	0.24739	0.29223	0.17139	0.1699	N																
CHD123	0.20649	0.21013	0.21013	0.24739	0.29223	0.17139	0.1699	0	N															
POR169	0.20649	0.21013	0.21013	0.24739	0.29223	0.17139	0.1699	0	0	N														
CHD118	0.20649	0.21013	0.21013	0.24739	0.29223	0.17139	0.1699	0	0	0	N													
TKS128	0.20813	0.21162	0.21162	0.24888	0.29376	0.17188	0.17139	0.00149	0.00149	0.00149	0.00149	N												
POR94	0.20483	0.20864	0.21162	0.24888	0.29249	0.1699	0.16841	0.00149	0.00149	0.00149	0.00149	0.00298	N											
CHD121	0.20483	0.20864	0.21162	0.24888	0.29249	0.1699	0.16841	0.00149	0.00149	0.00149	0.00149	0.00298	0	N										
GIL42	0.20819	0.21162	0.21461	0.25037	0.29548	0.17437	0.17288	0.00745	0.00745	0.00745	0.00745	0.00745	0.00596	0.00596	N									
SEM155	0.2048	0.20864	0.20864	0.2459	0.29068	0.17288	0.17139	0.00298	0.00298	0.00298	0.00298	0.00447	0.00447	0.00447	0.01043	N								
POR24	0.20479	0.20864	0.21162	0.25037	0.2925	0.17139	0.1699	0.00596	0.00596	0.00596	0.00596	0.00745	0.00447	0.00447	0.01043	0.00596	N							
POR26	0.20647	0.21013	0.21013	0.24888	0.29223	0.17288	0.17139	0.00447	0.00447	0.00447	0.00447	0.00596	0.00596	0.00596	0.01192	0.00447	0.00149	N						
LAB80	0.20788	0.21013	0.21311	0.23547	0.30097	0.16692	0.16542	0.13413	0.13413	0.13413	0.13413	0.13562	0.13562	0.13562	0.14158	0.13264	0.13413	0.13264	N					
LAB84	0.20788	0.21013	0.21311	0.23547	0.30097	0.16692	0.16542	0.13413	0.13413	0.13413	0.13413	0.13562	0.13562	0.13562	0.14158	0.13264	0.13413	0.13264	0	N				
LAB75	0.20788	0.21013	0.21311	0.23547	0.30097	0.16692	0.16542	0.13413	0.13413	0.13413	0.13413	0.13562	0.13562	0.13562	0.14158	0.13264	0.13413	0.13264	0	0	N			
RAM65	0.20788	0.21013	0.21311	0.23547	0.30097	0.16692	0.16542	0.13413	0.13413	0.13413	0.13413	0.13562	0.13562	0.13562	0.14158	0.13264	0.13413	0.13264	0	0	0	N		
LAB82	0.20788	0.21013	0.21311	0.23547	0.30097	0.16692	0.16542	0.13413	0.13413	0.13413	0.13413	0.13562	0.13562	0.13562	0.14158	0.13264	0.13413	0.13264	0	0	0	0	N	
LAB46	0.20788	0.21013	0.21311	0.23547	0.30097	0.16692	0.16542	0.13413	0.13413	0.13413	0.13413	0.13562	0.13562	0.13562	0.14158	0.13264	0.13413	0.13264	0	0	0	0	0	N
RAM47A	0.20954	0.21162	0.21461	0.23696	0.30251	0.16542	0.16393	0.13264	0.13264	0.13264	0.13264	0.13413	0.13413	0.13413	0.14009	0.13413	0.13562	0.13413	0.00149	0.00149	0.00149	0.00149	0.00149	0.00149
CHE141	0.21618	0.2161	0.22504	0.24143	0.28333	0.16095	0.15946	0.12966	0.12966	0.12966	0.12966	0.13115	0.12817	0.12817	0.13264	0.12817	0.12668	0.12817	0.14009	0.14009	0.14009	0.14009	0.14009	0.14009
CHE143C	0.21618	0.2161	0.22504	0.24143	0.28333	0.16095	0.15946	0.12966	0.12966	0.12966	0.12966	0.13115	0.12817	0.12817	0.13264	0.12817	0.12668	0.12817	0.14009	0.14009	0.14009	0.14009	0.14009	0.14009
SAY134	0.21618	0.2161	0.22504	0.24143	0.28333	0.16095	0.15946	0.12966	0.12966	0.12966	0.12966	0.13115	0.12817	0.12817	0.13264	0.12817	0.12668	0.12817	0.14009	0.14009	0.14009	0.14009	0.14009	0.14009
SAY135	0.21618	0.2161	0.22504	0.24143	0.28333	0.16095	0.15946	0.12966	0.12966	0.12966	0.12966	0.13115	0.12817	0.12817	0.13264	0.12817	0.12668	0.12817	0.14009	0.14009	0.14009	0.14009	0.14009	0.14009
CHE139B	0.21618	0.2161	0.22504	0.24143	0.28333	0.16095	0.15946	0.12966	0.12966	0.12966	0.12966	0.13115	0.12817	0.12817	0.13264	0.12817	0.12668	0.12817	0.14009	0.14009	0.14009	0.14009	0.14009	0.14009
CHE137	0.21618	0.2161	0.22504	0.24143	0.28333	0.16095	0.15946	0.12966	0.12966	0.12966	0.12966	0.13115	0.12817	0.12817	0.13264	0.12817	0.12668	0.12817	0.14009	0.14009	0.14009	0.14009	0.14009	0.14009
USA37	0.23994	0.25335	0.24441	0.23994	0.321	0.2608	0.25931	0.26528	0.26528	0.26528	0.26528	0.26677	0.26677	0.26677	0.27124	0.26677	0.26975	0.26826	0.26826	0.26826	0.26826	0.26826	0.26826	0.26826
MER20	0.36761	0.37109	0.35917	0.36066	0.38334	0.36215	0.36066	0.35768	0.35768	0.35768	0.35768	0.35917	0.35917	0.35917	0.36215	0.35768	0.36066	0.35917	0.35469	0.35469	0.35469	0.35469	0.35469	0.35469
GIL45	0.36761	0.37109	0.35917	0.36066	0.38334	0.36215	0.36066	0.35768	0.35768	0.35768	0.35768	0.35917	0.35917	0.35917	0.36215	0.35768	0.36066	0.35917	0.35469	0.35469	0.35469	0.35469	0.35469	0.35469
GIL57A	0.36761	0.37109	0.35917	0.36066	0.38334	0.36215	0.36066	0.35768	0.35768	0.35768	0.35768	0.35917	0.35917	0.35917	0.36215	0.35768	0.36066	0.35917	0.35469	0.35469	0.35469	0.35469	0.35469	0.35469
GIL89	0.36761	0.37109	0.35917	0.36066	0.38334	0.36215	0.36066	0.35768	0.35768	0.35768	0.35768	0.35917	0.35917	0.35917	0.36215	0.35768	0.36066	0.35917	0.35469	0.35469	0.35469	0.35469	0.35469	0.35469
RAM47B	0.36752	0.3696	0.35768	0.35917	0.3818	0.36066	0.35917	0.35618	0.35618	0.35618	0.35618	0.35768	0.35768	0.35768	0.36066	0.35618	0.35917	0.35768	0.3532	0.3532	0.3532	0.3532	0.3532	0.3532
SEM146	0.36605	0.37109	0.35618	0.35618	0.3788	0.35768	0.35618	0.35768	0.35768	0.35768	0.35768	0.35917	0.35917	0.35917	0.36215	0.35768	0.36066	0.35917	0.35469	0.35469	0.35469	0.35469	0.35469	0.35469
SEM152E	0.35094	0.36066	0.35618	0.35917	0.3772	0.37407	0.37258	0.35022	0.35022	0.35022	0.35022	0.35171	0.35171	0.35171	0.35469	0.35022	0.3532	0.35171	0.36066	0.36066	0.36066	0.36066	0.36066	0.36066
SEM160C	0.35629	0.36513	0.35618	0.36215	0.38197	0.3696	0.36811	0.34873	0.34873	0.34873	0.34873	0.35022	0.35022	0.35022	0.3532	0.34873	0.35171	0.35022	0.36215	0.36215	0.36215	0.36215	0.36215	0.36215
LAB53	0.35305	0.34724	0.34575	0.36811	0.3971	0.37854	0.38003	0.35171	0.35171	0.35171	0.35171	0.3532	0.3532	0.3532	0.35678	0.35171	0.35469	0.3532	0.37258	0.37258	0.37258	0.37258	0.37258	0.37258

Appendix D (continued)

	RAM47A	CHE141	CHE143C	SAY134	SAY135	CHE139B	CHE137	USA37	MER20	GIL45	GIL57A	GIL89	RAM47B	SEM146	SEM152E	SEM160C	LAB53
RAM47A	N																
CHE141	0.14158	N															
CHE143C	0.14158	0	N														
SAY134	0.14158	0	0	N													
SAY135	0.14158	0	0	0	N												
CHE139B	0.14158	0	0	0	0	N											
CHE137	0.14158	0	0	0	0	0	N										
USA37	0.26677	0.26677	0.26677	0.26677	0.26677	0.26677	0.26677	N									
MER20	0.35469	0.34575	0.34575	0.34575	0.34575	0.34575	0.34575	0.36662	N								
GIL45	0.35469	0.34575	0.34575	0.34575	0.34575	0.34575	0.34575	0.36662	0	N							
GIL57A	0.35469	0.34575	0.34575	0.34575	0.34575	0.34575	0.34575	0.36662	0	0	N						
GIL89	0.35469	0.34575	0.34575	0.34575	0.34575	0.34575	0.34575	0.36662	0	0	0	N					
RAM47B	0.3532	0.34426	0.34426	0.34426	0.34426	0.34426	0.34426	0.36513	0.00149	0.00149	0.00149	0.00149	N				
SEM146	0.35469	0.34426	0.34426	0.34426	0.34426	0.34426	0.34426	0.36215	0.01043	0.01043	0.01043	0.01043	0.01192	N			
SEM152E	0.36066	0.34873	0.34873	0.34873	0.34873	0.34873	0.34873	0.3532	0.07154	0.07154	0.07154	0.07154	0.07303	0.07303	N		
SEM160C	0.36215	0.34873	0.34873	0.34873	0.34873	0.34873	0.34873	0.34873	0.07004	0.07004	0.07004	0.07004	0.07154	0.07154	0.00894	N	
LAB53	0.37258	0.36215	0.36215	0.36215	0.36215	0.36215	0.36215	0.34873	0.1386	0.1386	0.1386	0.1386	0.14009	0.13711	0.14754	0.14754	N

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Mesospora elongata sp. nov. (Ralfsiales, Phaeophyceae), a new crustose brown algal species from the Indo-Pacific region

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Crustose brown algae have relatively simple morphology and anatomy, and the limited characters were a major obstacle in delineating species of *Mesospora* (Ralfsiales, Phaeophyceae). The type species, *Mesospora schmidtii*, was described using material collected in Indonesia. A subsequent study, using material from Japan, was identified as *M. schmidtii*, but we questioned the identity. Therefore, we analyzed morphological characters as well as the plastid-encoded RuBisCO large subunit (*rbc*L) and mitochondrial-encoded cytochrome *c* oxidase subunit 1 (*cox*1) genes using new material obtained from the type locality (Indonesia) and new material obtained from Japan. The molecular phylogenetic analysis distinguished two clades, one representing material from the type location and the other representing an undescribed species from Japan. Therefore, we described *Mesospora elongata* sp. nov. to encompass the Japanese material. Morphologically, the two species were very similar (i.e. cryptic species); the only obvious difference was the number of cells. We also discussed *Hapalospongidion* but we concluded that *Mesospora* should be a distinct genus based on morphology; gene sequence data will be required for *Hapalospongidion* before the relationship of the two genera is more thoroughly resolved.

Key Words: *cox*1, Cryptic species, *Mesospora elongata*, *Mesospora schmidtii*, Ralfsiales, *rbc*L

INTRODUCTION

The marine crustose brown algal genus *Mesospora* was first described by Weber-van Bosse (1911) on the basis of materials collected from several localities in Indonesia during the Siboga Expedition. Weber-van Bosse (1911, 1913) initially placed *Mesospora* in the family Ralfsiaceae with *M. schmidtii Weber-van Bosse* as the generitype albeit with some uncertainty in view of the thin horizontal basal layer of the thallus, which consisted of generally two and rarely four layers of cells. Other generic characteristics provided by the author include (1) a mucilaginous thallus wholly adherent to the substratum, (2) loosely adherent clavate erect filaments, (3) intercalary plurilocular sporangia near the apex of filaments and (4) ovate, unilocular sporangia laterally borne at the base of filaments. *Mesospora* has been considered as a synonym of *Hapalospongidion* (Womersley 1987; León-Alvarez & González-González 1993; Silva *et al*. 1996); however, based upon morphological details, we consider the genus *Mesospora* to be distinct from *Hapalospongidion* (see Discussion).

Currently, five species of *Mesospora* are recognised (Guiry & Guiry 2012): *M. schmidtii* Weber-van Bosse (1911) from Indonesia is the type species, *M. vanbosseae* Børgesen (1924) from Easter Island, *M. macrocarpa* (Feldmann) den Hartog (1968) from the Mediterranean, *M. pangoensis* (Setchell) Chihara & Tanaka from Tutuila Island and its variety *M. pangoensis* var. *galapagensis* (Setchell & Gardner) Chihara & Tanaka (Tanaka & Chihara 1982) from the Galapagos Islands, and *M. negrosensis* West & Calumpong (1996) from the Philippines. The known distribution of *M. schmidtii* is confined to the warmer waters of the Indian Ocean and the (sub)tropical western Pacific Ocean. In addition to the original description, *M. schmidtii* has been reported in Vietnam (Dawson 1954), Hong Kong (Kaehler 1994), Japan (Tanaka & Chihara 1982; Yoshida *et al*. 1990), the Solomon Islands (Womersley & Bailey 1970), Australia (Phillips 2002) and Malaysia (Lim *et al*. 2008). A detailed study by Tanaka & Chihara (Tanaka & Chihara 1982) led to the establishment of the family Mesosporaceae to accommodate *Mesospora*, *Hapalospongidion* and *Basispora*. However, a number of discrepancies in the descriptions of *M. schmidtii* by Weber-van Bosse (1911, 1913) and Tanaka & Chihara (1982) suggest that the two may be distinct entities. The present study addresses the issue using morphological observations and molecular analysis of the plastid RuBisCO large subunit (*rbc*L) and mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) genes. We recollected *M. schmidtii* specimens from Batukijok, near the syntype locality: bay of Labuan Terang [Labuan Tring in Weber-van Bosse (1911, 1913)], Lombok Island, Indonesia. *Mesospora schmidtii* specimens were also collected from Malaysia.

MATERIAL AND METHODS

Brown algal crusts on rocks were collected from Lombok Island, Indonesia; Malaysia; and Ishigaki Island, Japan.

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Smaller rocks were air-dried prior to desiccation in silica gel; they were maintained at ambient temperature. Rocks too large to be transported back to the laboratory were collected by either breaking off pieces of the rock or scraping crusts from the rock with razor blades. Specimens were deposited in the University of Malaya Seaweeds and Seagrasses Herbarium and the herbarium of the Kobe University Research Center for Inland Seas (KURCIS). Gene sequences were either newly generated or retrieved from the GenBank (Table S1).

Light microscope observations were made using squash preparations mounted in corn syrup. Characters examined included number of cells, cell size, position and dimensions of unilocular and plurilocular sporangia, number of sterile terminal cell(s), number of stalk cell(s) and length of erect filament.

Approximately 3–5 mg of dried thalli was ground in liquid nitrogen and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The *rbc*L gene (approximately 1340 nucleotides) was amplified as two or three overlapping fragments using the primer sets NDrbcL2-NDrbcL9 and PRBF3-PRBR3 or rbcF0-RalR952, PRBF2-PRBR2 and PRBF3-PRBR3. The first half of the *cox*1 gene (approximately 650 nucleotides) was also sequenced. PCR amplification was carried out using MultiGene Thermal Cycler TC9600-G (Labnet International Inc., USA). The total volume was 20 µl: 2 µl of 10x *i*-Taq plus reaction buffer, 0.75 µl of dNTP mixture (consisting of 2.5 mM each of dNTP), 1 µl of each forward and reverse primers (10 pmol/µl) (see Table S2), 0.25 µl of *i*-Taq plus DNA polymerase (iNtRON Biotechnology, Korea), 1 µl (25–50 ng) genomic DNA and 14 µl ultrapure water. Using a modified PCR routine for the amplification of the *rbc*L gene with the primer set NDrbcL2/NDrbcL9, an initial denaturation for 3 min at 94°C was followed by five cycles of denaturation at 94°C for 30 s, annealing at 47°C for 30 s, extension at 72°C for 1 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 1 min; and a final extension at 72°C for 10 min. Thermal cycling performed using other primer pair combinations followed Bittner *et al*. (2008) but used an annealing temperature of 50°C for *rbc*L amplifications (see Table S2 for *cox*1). PCR products were checked for length and yield by electrophoresis on 1% agarose gels stained with SYBR Safe DNA gel stain (Invitrogen, USA). The resulting products were purified using LaboPass Gel and PCR Clean-up kit (Cosmo Genetech, South Korea) and sequenced commercially by First BASE (http://www.base-asia.com).

The raw sequences were assembled and edited in the program ChromasPro ver. 1.42 (Technelysium Pty Ltd, Australia). Consensus sequences were preliminarily aligned in ClustalX v. 2.0.8 (Larkin *et al*. 2007) and subsequently manually revised in Bioedit v. 7.0.9.0 (Hall 1999). Two data sets were employed for the phylogenetic analyses: 13 taxa for *rbc*L and 10 taxa for *cox*1–5′. *Ralfsia fungiformis* (Gunnerus) Setchell & Gardner and *Analipus japonicus* (Harvey) Wynne sequences for both genes were obtained from GenBank and used as outgroup taxa (Table S1).

Maximum Parsimony (MP) analyses were accomplished in PAUP* 4.0b10 (Swofford 2001) using heuristic search with 100 random sequence addition replicates and a tree bisection reconnection (TBR) branch-swapping algorithm. Gaps in the alignment were treated as missing data. All characters were treated as unordered and equally weighted, the Multrees option active and branches with a maximum length of zero collapsed to yield polytomies. To assess support for the resulting nodes, bootstrap percentage (BP) was computed with 1000 replications using one random taxon additions under the heuristic search method with TBR swapping.

Prior to Maximum Likelihood (ML) analyses using PAUP* 4.0b10 (Swofford 2001), the program Modeltest v.3.7 (Posada & Crandall 1998) was used to determine the sequence evolution model that best fits our data, using the hierarchical likelihood ratio test (hLRT) ($a = 0.01$). For the *rbc*L data set, the best model selected was a general time reversible (GTR) model with a gamma distribution. The chosen model for *cox*1–5′ data set was the HKY + G model. One hundred random sequence addition replicates were analysed under the heuristic search algorithm, and subsequently 100 bootstrap replicates were performed with one random sequence addition replicates.

Kakusan v.3 (Tanabe 2007) was used to select the optimal model of sequence evolution to fit our data based on Bayesian Information Criterion (BIC) for Bayesian Inference (BI) analyses. Phylogenetic trees were then constructed using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). Bayesian analyses for both data sets were performed using the GTR + Gamma model. Two parallel independent runs of four Markov chains were performed for 2,000,000 generations and trees were sampled every 100th generation. Log likelihood values reached a plateau within the first 20,000 generations in both *rbc*L and *cox*1–5′ analyses. To ensure stabilization, the first 200 trees were discarded as burn-in and the remaining trees of both runs were used to compute the consensus trees. Node credibility of the BI tree was evaluated by Bayesian posterior probabilities (PP).

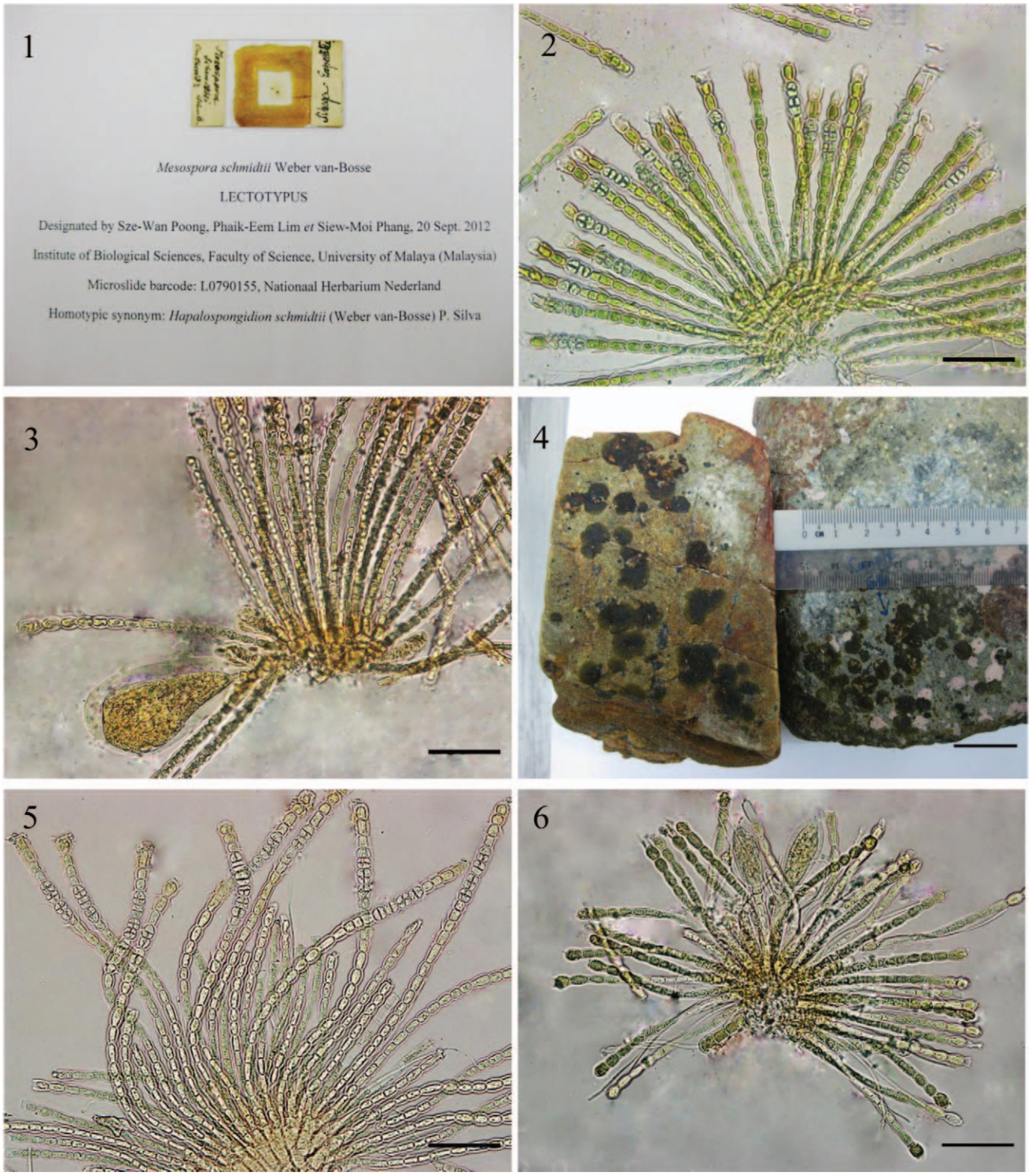
For bootstrapping comparison purposes, nodal support was deemed strong for those with $BP \geq 85\%$ and $PP > 0.95$, moderate for $70\% \leq BP < 85\%$ and $0.90 \leq PP \leq 0.95$ and weak for $BP < 70\%$ and $PP < 0.90$. To assess the level of intra- and interspecific variations in the *rbc*L and *cox*1 sequences, uncorrected (*p*) pairwise genetic distances were estimated using PAUP* 4.0b10 software (Swofford 2001).

RESULTS

Mesospora schmidtii Weber-van Bosse

Figs 1–3

In the protologue, Weber-van Bosse (1911) did not designate a holotype among specimens from the original six syntype localities. Here we designated as lectotype of *Mesospora schmidtii* Weber-van Bosse the microslide L0790155 (Fig. 1) held in the Herbarium of Weber-van Bosse, included in the Nationaal Herbarium Nederland (L). We chose this material because it was part of the original material of this taxon (ICBN, Art. 9.2, Note 2) and corresponded to the drawings that Weber-van Bosse included in her later publication (Weber-van Bosse 1913: 143–145, fig. 43 and pl. II, figs. 2, 3). Annotations on the microslide: 'Siboga-Expeditie,' '*Mesospora*



Figs 1–3. *Mesospora schmidtii*.
Fig. 1. Image of the lectotype of *M. schmidtii*, microslide L0790155 (L, in Herbarium Weber-van Bosse) with annotations.
Fig. 2. Squash preparation of *M. schmidtii* showing erect filaments bearing plurilocular sporangia with sterile terminal cells. Voucher number = PSM 12207. Slide PSM 12207-96-1. Scale bar = 50 μm.
Fig. 3. Squash preparation of *M. schmidtii* showing a unilocular sporangium terminally borne on stalk cells adjacent to longer erect vegetative filaments. Voucher number = PSM 12246. Slide PSM 12246-GB7A-2. Scale bar = 50 μm.
Figs 4–6. *Mesospora elongata* sp. nov.
Fig. 4. Crusts of *Mesospora elongata* sp. nov. on rocks. Scale bar = 2 cm.
Fig. 5. Squash preparation of *Mesospora elongata* sp. nov. showing conspicuously longer erect filaments bearing plurilocular reproductive structures with sterile terminal cells. Voucher number = KU-d11206 (holotype). Slide KU-d112206-FUS3-1. Scale bar = 50 μm.
Fig. 6. Squash preparation of *Mesospora elongata* sp. nov. showing unilocular sporangia terminally borne on stalk cells, lateral to the erect vegetative filaments. Voucher number = PSM 12221. Slide PSM 12221-38-1. Scale bar = 50 μm.

Table 1. Summary of statistics from MP analyses of *rbc*L and *cox*1 data sets.

	*rbc*L	*cox*1–5′
No. of taxa	13	10
Length of alignment	1340	657
No. of parsimony informative characters	203 (15%)	200 (30.5%)
No. of variable uninformative characters	105 (8%)	56 (8.5%)
No. of constant characters	1032 (77%)	401 (61%)
No. of MP trees	1	4
MP tree length	494	462
Consistency index	0.7206	0.7900
Retention index	0.7625	0.7954

schmidtii Weber-van B.' and 'antheridia' were in her own writings. An epitype was also designated (ICBN, Art. 9.7) because the exact type locality for the lectotype was unknown although all syntype localities were in Indonesia. After careful consideration, microslide PSM 12246-GB7A-1, housed at the University of Malaya Seaweeds and Seagrasses Herbarium, was designated as epitype of *M. schmidtii* in support of the above designated lectotype (L0790155). The epitype was collected from a distinct geographic point near to one of the syntype localities. Epitype collection details are: Batukijok, Lombok Island, Indonesia (*P.-E. Lim*, 1 May 2006, PSM 12246-GB7A-1).

HOMOTYPIC SYNONYM: *Hapalospongidion schmidtii* (Weber-van Bosse) P.C. Silva.

TYPE LOCALITY: Indonesia (Siboga Expedition).

SPECIMENS EXAMINED: Batukijok, Lombok Island, Indonesia, 1 May 2006 (PSM 12246), 10 June 2010 (PSM 12235); Pulau Che Kamat, Johor, Malaysia, 29 May 2009 (PSM 12173); Port Dickson, Negeri Sembilan, Malaysia, 16 December 2009 (PSM 12201, PSM 12203, PSM 12207); Pulau Merambong, Johor, Malaysia, 24 August 2009 (PSM 12179, PSM 12187, PSM 12197); Tanjung Langsat, Johor, Malaysia, 27 May 2010 (PSM 12211); Kampong Dandulit, Sabah, Malaysia, 9 November 2010 (PSM 12244, PSM 12245).

The plants were epilithic and formed smooth (at times, flaky), dark brown-black crusts; crusts were sticky upon contact with water. Thalli were firmly adherent to the rock substrate over the entire under surface, i.e. there were no rhizoids. The crust outline was discrete and orbicular in juvenile plants; the crust often became irregular in older thalli as a result of confluence with surrounding thalli. In squash preparations, the thallus comprised a horizontal basal layer of one to four (generally two) layers of adjoined rectangular cells. The basal region gave rise to erect, free, simple and unbranched filaments that were composed of 10–19 cells. These loosely associated filaments, held within a gelatinous matrix, were readily separated under light pressure. The erect filaments were slightly clavate in appearance. Cells at the basal end of the filaments were cylindrical; cells measured 7.2–14.8 (–17.7) μm in length and 2.8–6.8 (–10.0) μm in width; the length to diameter ratio was 1.5–3.5. The upper region cells were subglobose, and they were 5.7–10.7 (–13.2) μm in length and 4.3–8.8 (–11.2) μm in width; the length to diameter ratio was between 0.8:1 to 2.5:1. Intercalary plurilocular sporangia occurred in the upper portion of erect filaments, and the sporangia terminated with two to three sterile terminal cells. Plurilocular sporangia were 23.3–33.5 (–42.9) μm in length, 6.6–10.0 (–12.3) μm in width; the locules were somewhat regularly arranged. Unilocular sporangia were terminally inserted on stalks of up to four cells; they were lateral and basal to the surrounding filaments. The unilocular sporangia were 63.3–143.2 μm in length and 20.2–48.8 μm in diameter. The stalk cells were shorter than other cells of the vegetative filaments. The two types of sporangia were not conclusively found on the same plant; it was difficult to separate confluent individuals because of the continuous crust. Infrequently, colourless hairs emerged as tufts from depressions in the basal layer. Sheath-like cell wall remnants were attached prominently above and/or below the plurilocular sporangia; occasionally sheath-like remnants were attached to sterile terminal cells and other vegetative cells.

In addition, we examined specimens that were distinguished from *Mesospora schmidtii* (Figs 4–6). These plants had erect filaments that were comprised of 11–29 cells. Cells near the basal end of the erect filaments were 6.3–15.9 (–23.7) μm in length and 2.5–6.0 (–9.2) μm in width; cells at the distal end were 5.8–10.7 (–17.1) μm in length and 3.4–7.9 (–13.2) μm in width. Plurilocular sporangia were (13.8) 22.1–49.9 μm in length, (4.8) 8.1–10.9 μm in width, and some of the locules were uniseriate. Unilocular sporangia were 25.9–76.7 μm in length and 11.5–26.9 μm in diameter; they were terminally inserted on stalks of up to eight cells, lateral to the surrounding filaments.

Molecular phylogenetic analyses

Mitochondrial *cox*1 sequences were more variable and more parsimony informative than plastid *rbc*L sequences (see Table 1). For the *rbc*L sequences, all three methods of phylogenetic inference (ML, MP, BI) yielded congruent, though not identical trees; only the ML tree is shown (Fig. 7). Two strongly supported clades were recovered (clade A and clade B, Fig. 7), and we interpreted this as evidence for two independent species. The basic topologies of the ML and MP trees were similar, and they also resolved a strongly monophyletic clade of *Mesospora* species (100% BP for ML and MP). The only difference between the ML and MP trees was the reverse branching order of clade B and the *Mesospora* sp. C/ *Mesospora* sp. D clade. *Mesospora* sp. G was separated from the rest of the *Mesospora* cluster in the BI tree. Despite that, support for the *Mesospora* cluster excluding *Mesospora* sp. G was strong with PP = 1 (100% BP for ML and MP). Sequences within clade B were more divergent with 6–19 bp (0.45%–1.42%) differences whereas sequences within clade A were identical. Meanwhile the variation between the two clades was 102–116 bp (7.74%–8.66%).

The results of the ML, MP and BI analyses of the *cox*1–5′ alignments are summarized in Fig. 8. Consistent with *rbc*L analyses, the *Mesospora* taxa were monophyletic with strong support (BP = 100%) in the ML and MP trees but this relationship was not resolved in the BI tree. The *Mesospora* taxa were likewise split into two major clades, A and B (Fig.

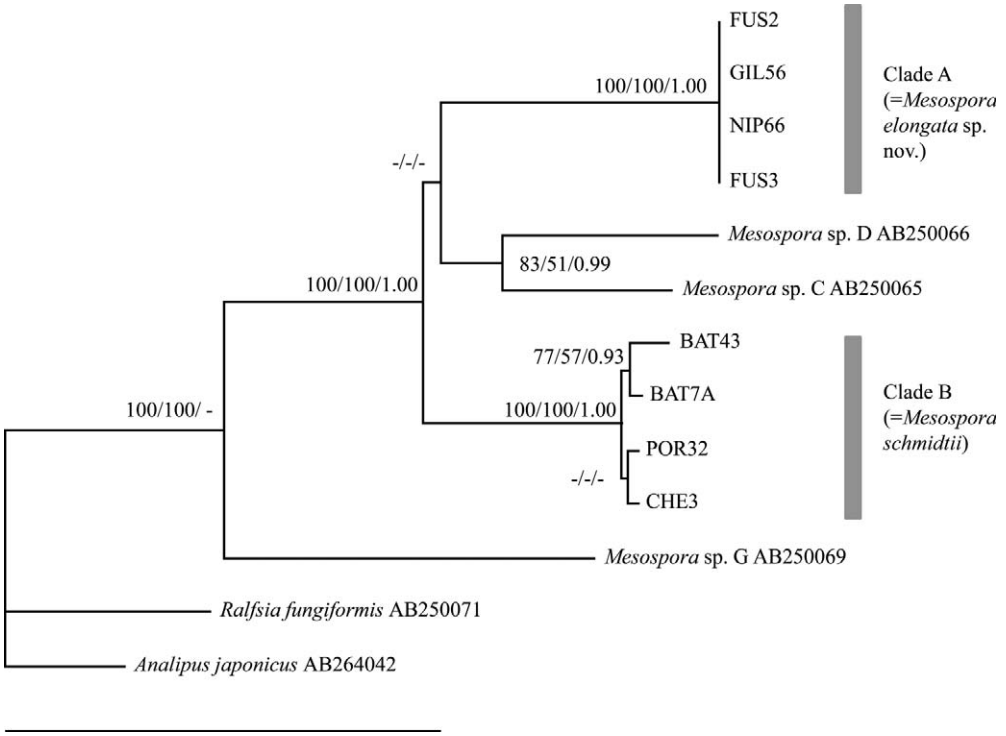


Fig. 7. ML phylogeny inferred based on *rbc*L gene sequences. Numbers associated with each branch indicate bootstrap values (> 50%) for ML (left), MP (middle) and BI (right). Dashes (-) denote percentages of <50% (or that the internode did not occur in the MP or BI tree). –Ln likelihood was 4124.3086; gamma distribution shape parameter (alpha) = 0.1704; nucleotide frequencies: A = 0.2833, C = 0.1667, G = 0.2325, T = 0.3175 and substitution model rate matrix: (R(a) [A-C]: 0.7755, R(b) [A-G] = 9.3795, R(c) [A-T] = 2.8709, R(d) [C-G] = 1.1704, R(e) [C-T] = 23.0905, R(f) [G-T] = 1.0000). Scale bar = 0.1 substitutions per site.

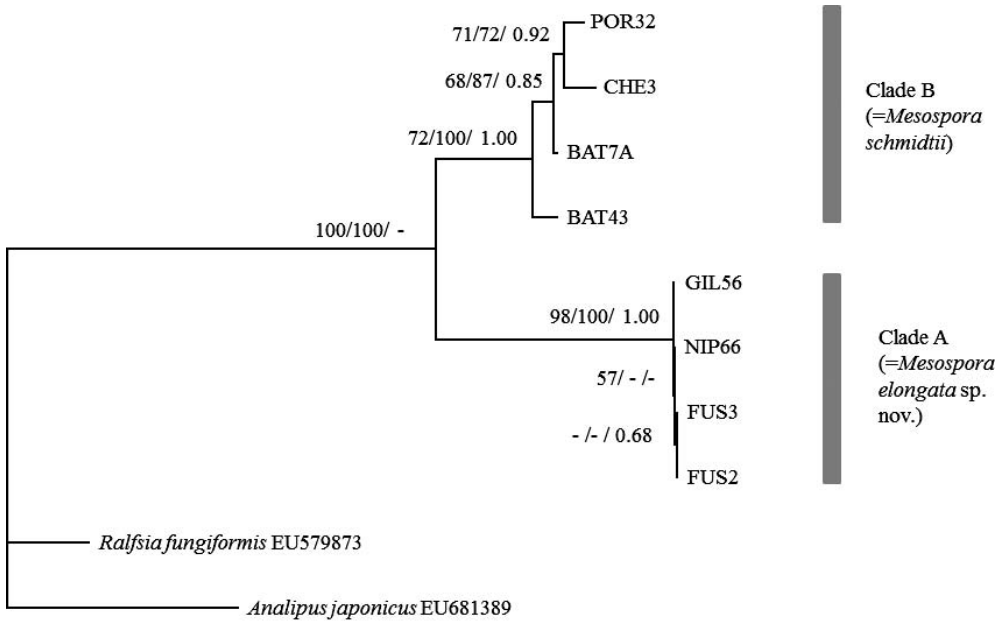


Fig. 8. ML phylogeny inferred based on *cox*1–5′ gene sequences**.** Numbers associated with each branch indicate bootstrap values (> 50%) for ML (left), MP (middle) and BI (right). Dashes (-) denote percentages of <50% (or that the internode did not occur in the MP or BI tree). –Ln likelihood was 2680.3987; gamma distribution shape parameter (alpha) = 0.1528; nucleotide frequencies: A = 0.2095, C = 0.1897, G = 0.1941, T = 0.4068; Ti/tv ratio = 5.9171. Scale bar = 0.1 substitutions per site.

8). Clade A received strong support from all three analyses (98/100% BP for ML/MP, and PP = 1 for BI). Clade B was moderately supported in ML (BP = 72%) but it received strong support from MP (BP = 100%) and BI (PP = 1). ML, MP and BI trees were identical in topology except for the branching order. The uncorrected sequence divergences (*p*-distance) within clades A and B were 0–2 bp (0%–0.30%) and 43–81 bp (6.54%–12.33%) respectively; those between the two clades were 131–145 bp (19.94%–22.07%).

DISCUSSION

The status of the genus *Mesospora* has received considerable attention. Womersley (1987) regarded *Mesospora* and *Basispora* to be closely related to *Hapalospongidion* or even placed in synonymy with the latter genus. He based this conclusion on chloroplast number and position of unilocular sporangia; specifically, *Mesospora* and *Hapalospongidion* have one to three chloroplasts per cell and unilocular sporangia occur terminally on vegetative filaments. *Basispora* is similar; it has several chloroplasts per cell and terminal unilocular sporangia arise on long stalks from near the base of the laterally free erect filaments. Others have pointed out the close relationship among the genera: all include crustose plants with a base of prostrate filaments from which simple, loosely adjoined filaments arise; all have intercalary plurilocular sporangia and terminal unilocular sporangia arising on short erect filaments (John & Lawson 1974; Tanaka & Chihara 1982). Womersley (1987) also placed *Hapalospongidion* in the Ralfsiaceae instead of Mesosporaceae, but a molecular study showed that the two families were separate (Lim *et al*. 2007).

Hapalospongidion was described as a gelatinous thallus comprising erect, unbranched filaments arising from a distromatic basal plate (Saunders 1899). There were two types of erect filaments; intercalary plurilocular sporangia were formed on the longer filaments and unilocular sporangia were terminally formed on the shorter filaments. The illustration by Saunders (1899: pl. I, fig. 2b and 2b′) illustrated two peculiar structures on the shorter filaments: one was a series of enlarged cells described as rare and immature or abortive unilocular sporangia, the other was a few lateral outgrowths at the apex of the filament. However, Hollenberg (1942) commented that his specimens, collected from the type locality, did not show any seriate intercalary unilocular sporangia as reported by Saunders (1899) and Hollenberg dismissed the structure as abortive plurilocular sporangia. Furthermore, Hollenberg interpreted the lateral outgrowths as aborted accessory unilocular sporangia or the true tip of the fruiting filament. Significantly, neither of these two structures has been reported for *Mesospora* or *Basispora*. There is another important distinction between *Mesospora* and *Hapalospongidion*, often overlooked: terminal unilocular sporangia arise directly from the basal layer on shorter erect filaments in *Hapalospongidion* but terminal unilocular sporangia arise on a morphologically differentiated stalk cell or cells that are issued laterally from erect filaments in *Mesospora* (and not necessarily basal in position, e.g. *M. mesocarpa*). We did observe unilocular sporangia occurring on thallus with fewer cell numbers per filament but because of the scarcity of unilocular sporangia in our collections, we remain uncertain about this observation. *Mesospora* is also distinguished from *Hapalospongidion* by its vegetative filaments, which are considerably shorter (less than 35 cells) (Womersley 1987). Furthermore, *Hapalospongidion* has a soft and cushion-like thallus (see Saunders 1899: pl. I, fig. 1 and Hollenberg 1942) and reflected in its name derived from the Greek words '*apalos*' and '*spongos*' meaning soft and sponge, respectively. Conversely, the thallus of *Mesospora* is not sponge-like. *Basispora* was characterised as having terminal unilocular sporangia on distinct stalks that emerge laterally from near the base of erect filaments, several discoid chloroplasts per cell, and no known plurilocular sporangia (John & Lawson 1974); therefore, *Basispora* is distinguished from *Hapalospongidion* by the position of the unilocular sporangia, which are laterally placed on erect filament at the end of stalk cells (in *Hapalospongidion*, they are terminal on the erect filament itself). Based upon these features, we retain *Mesospora* and *Basispora* as genera distinct from *Hapalospongidion*. It is evident that further investigation is necessary to clarify the taxonomic status of the three genera.

The high degree of morphological similarity between our *Mesospora schmidtii* specimens and our specimens that match the description of *M. schmidtii sensu* Tanaka & Chihara (1982) (clade A, Figs 7, 8) suggests that these represent two cryptic species that are clearly separated using molecular phylogenetic analyses. Nevertheless, both groups of specimens were distinguished using cell numbers in filaments. For example, both clades were present on Lombok Island; using squash preparations of *M. schmidtii* from Batukijok, we distinguished *M. schmidtii* from specimens of clade A. Clade A possessed longer erect filaments composed of 11–29 cells, which fitted the description of *M. schmidtii* given by Tanaka & Chihara (1982). We agree with Tanaka & Chihara (1982) that the plurilocular sporangia observed by Weber-van Bosse (1911, 1913) had not reached full maturity, i.e. we found sporangia with more than eight locules from Batukijok. The size of plurilocular sporangia was not given by Weber-van Bosse (1911, 1913) and no size comparison could be made. The size of plurilocular sporangia in our specimens of *M. schmidtii* (clade B, Figs 7, 8) were smaller [23.3–33.5 (–42.9) μm in length, 6.6–10.0 (–12.3) μm in width] than those observed for our specimens in clade A (Figs 7, 8) [(13.8) 22.1–49.9 μm in length, (4.8) 8.1–10.9 μm broad]. Furthermore, plurilocular sporangia from clade A were smaller than those reported by Tanaka & Chihara (1982). Unilocular sporangia of our *M. schmidtii* specimens agreed well with the description by Weber-van Bosse (1911, 1913); however, they were broader and less elongate (ratio-wise) than those of the clade A specimens**.** Similar to plurilocular sporangia, clade A unilocular sporangia were smaller than those reported by Tanaka & Chihara (1982); this suggests that clade A unilocular sporangia had not reached full maturity. The discrepancies between our clade A specimens and those described by Tanaka & Chihara could also be attributed to small sample size caused by the scarcity of the specimens during the time of our collections.

Sheath-like remnants of old cell walls attached to the cells surrounding the plurilocular sporangia, occasionally on

vegetative filaments, were observed in both *M. schmidtii* and clade A specimens. The sheath-like feature was first clearly attributed to *Mesospora* by Tanaka & Chihara (1982); it was illustrated on the cells of vegetative filaments by Weber-van Bosse (1913). Weber-van Bosse's description was vague but close inspection of her illustrations showed sheath remnants near or surrounding the plurilocular sporangia. Our observations of sheath-like remnants on sterile terminal cells concurred with her description of apical cells, i.e. not smooth but with traces of membranes. We did not observe the sheath-like remnants of cell walls on the stalk cells of unilocular sporangia as reported by Tanaka & Chihara (1982).

Molecular phylogenetic analyses separated clades A and B (Figs 7, 8), and we consider the two clades independent taxa at the species level. Our study included only two of five currently recognised species of *Mesospora* because we did not obtain specimens from the type localities of the other three species. We included all available sequences from GenBank. Furthermore, based upon an unpublished *rbc*L sequence provided by John A. West and Joe Buchanan, we confirmed that none of our specimens were *M. negrosensis*. A comparison of species of *Mesospora* based on morphological data from the literature is also presented here (see Table S3).

The barcoding candidate and faster evolving *cox*1 gene (Hebert *et al*. 2003; McDevit & Saunders 2009) showed that clade B (*M. schmidtii*) has a surprisingly high within-species variation (Fig. 8). Nonetheless, there was no overlap between inter- and intraspecific divergence for *cox*1–5′ in our samples, further attesting to its efficiency as a barcoding marker; this was the first use of *cox*1 as a barcoding marker for the Ralfsiales.

Mesospora schmidtii and clade A are distinguished from *M. vanbosseae* because the latter has a thick basal layer consisting of ten or more layers, lacks unilocular sporangia, has cylindrical cells with thin walls, and an irregular arrangement of locules in the plurilocular sporangia (see Børgesen 1924, p. 259, fig. 9d). *Mesospora negrosensis* can be distinguished from clade A specimens (in addition to previously mentioned unpublished molecular data) because it has fewer cell numbers per filament, no unilocular sporangia and mostly uniseriate plurilocular sporangia (perhaps not fully matured). The position of unilocular sporangia of *M. mesocarpa*, which often arise laterally in the middle of an erect filament, is an important difference between this species and clade A specimens. *Mesospora pangoensis* differs from clade A specimens because it has fewer cells in erect filaments, unknown plurilocular sporangia, a smaller number of stalk cells (three to five cells), and much shorter and broader unilocular sporangia.

Based on molecular and morphological data, we describe clade A specimens as a new species.

Mesospora elongata S.W. Poong, P.E. Lim & S.M. Phang sp. nov.

DIAGNOSIS: Thallus crustose, thin, tightly adherent to the substratum without rhizoids, composed of thin (generally two) layers of horizontal basal cells wider than high and loosely adjoined erect filaments up to 30 cells long; gelatinous upon contact with water; unilocular sporangia lateral to surrounding filaments, with up to eight stalk cells, without paraphyses; intercalary biseriate plurilocular sporangia with two to four sterile terminal cells; *rbc*L and *cox*1 gene sequences distinct from other studied *Mesospora* species.

HOLOTYPE DESIGNATED HERE: KU-d11206, collected by *P.E. Lim* on 6 February 2005, Herbarium of the Kobe University Research Center for Inland Seas, Rokkodai, Kobe 657-8501, Japan.

DNA SEQUENCES FROM THE HOLOTYPE: JQ620003 (*rbc*L), JQ620011 (*cox*1).

TYPE LOCALITY: rocks, littoral zone, Fusaki, Ishigaki Island, Okinawa Prefecture, Japan.

ETYMOLOGY: *elongata* (Latin) refers to the long filaments arising from the thallus.

ISOTYPE: Nipah, Lombok, Indonesia, 8 June 2010 (PSM 12214).

PARATYPES: Fusaki, Ishigaki Island, Okinawa Prefecture, Japan, 6 February 2005 (KU-d11205); Gili Genting, Lombok, Indonesia, 10 June 2010 (PSM 12221, PSM 12225).

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at http://dx.doi.org/10.2216/12-42.1.s1.

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A molecular-assisted floristic survey of crustose brown algae (Phaeophyceae) from Malaysia and Lombok Island, Indonesia based on *rbc*L and partial *cox*1 genes

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Abstract Studies on the crustose brown algae are relatively few despite a long history of studies conducted since the 1800s, with temperate species forming the bulk of these studies. There is a need for more focus on crustose brown algae particularly in the tropics as they are generally different from those in the temperate regions. Taxonomic confusion arising from morphological simplicity largely dependent on the reproductive structures and overlap in morpho-anatomical features among species necessitates the use of molecular techniques. This study is dedicated to a better understanding of the diversity of these understudied algae in the Indo–Malay region. Specimens collected from Peninsular Malaysia, Sabah (Borneo) and Lombok Island in Indonesia were identified using molecular markers from the plastid rubisco large subunit (*rbc*L) and mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) genes in tandem with morphology and anatomy. Three *Mesospora* spp., two putative *Diplura* spp. and the cosmopolitan *Neoralfsia expansa* were identified in this study, including a new record of *Mesospora negrosensis* for Malaysia. Despite their morpho-anatomical similarities, *Mesospora* and *Diplura* occur in widely divergent clades within the brown algae, the former in the Mesosporaceae in the Ralfsiales, the latter in an unclassified clade sister to the Ishigeales. All six species occurred both in Malaysia and Lombok Island except for *M. elongata* and *M. negrosensis*, respectively. The *rbc*L marker performed better in the elucidation of phylogeny among the brown algal orders, whereas *cox*1-5′ is more suited as a barcoding marker for species level identification.

Keywords *Diplura* · Diversity · Indo–Malay region · *Mesospora* · *Mesospora negrosensis* · *Neoralfsia expansa* · New record · Ralfsiales

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Introduction

Weber-van Bosse (1911, 1913) initiated studies on crustose brown algal taxa in the Indo–Malay region using materials collected during the Siboga Expedition. Five taxa were identified in her studies and were placed in two families: *Neoralfsia expansa* (J. Agardh) Lim et Kawai ex Cormaci et G. Furnari (as *Ralfsia expansa* J. Agardh), *Mesospora schmidtii* Weber-van Bosse, *Stragularia clavata* (Harvey) G. Hamel (as *Stragularia clavata* (Carmichael) Kjellman) and *S. polycarpa* Weber-van Bosse in the Ralfsiaceae while a putative species of *Lithoderma* was placed in the Lithodermataceae. In Malaysia, the crustose brown algae were first documented by Phang et al. (2007) in which only the genus *Ralfsia* was recorded and no details on the description or distribution of the species was given. Subsequently *N. expansa*, *M. schmidtii* and *Mesospora* sp. C were reported in two publications on Ralfsiales in Malaysia (Lim et al. 2007, 2008).

The Indo–Malay archipelago, located between the Indian and Pacific Ocean, is well known as a marine biodiversity hotspot (Hoeksema 2007). Yet, there are relatively few reports of crustose brown algal taxa from this enclave. The

geographical coverage of the present study, which includes Peninsular Malaysia, Sabah (Borneo) and Lombok Island (Indonesia), is chosen for its location, the history of taxonomic work on crustose brown algal taxa and the manageable number of taxa. Lombok Island, which lies south to the equator (latitude 08° S), is also considered as a study site for a rough estimate of southern hemisphere crustose brown algal diversity. From the time of Weber-van Bosse's work until recently, only one new addition (i.e., *M. elongata* Poong, Lim et Phang; Poong et al. 2013) was made to the Indo–Malay crustose brown algal flora, clearly highlighting a need for this study.

A major challenge in the taxonomy of crustose brown algae is the difficulty in identifying species based solely on morphological and anatomical features. Like the red algae, their classification and taxonomy has largely relied on presence of reproductive structures. An instance of their hazardous identification is seen when Kain et al. (2010) misidentified the crustose form of *Colpomenia bullosa* (Saunders) Yamada and an unidentified species of *Ralfsia* as *Ralfsia verrucosa* (Areschoug) J. Agardh, and the misidentification was only realised upon conducting molecular analyses. Taxonomic and systematic studies on crustose brown algae began in the 1800s (e.g., Agardh 1847, p. 7), but the majority were based on conventional morphology description without the support of molecular data. Gene sequence data is currently used in combination with existing morphology observation to improve classification at higher taxonomic levels, estimates of species diversity, species delineation and knowledge of evolutionary relationships (Kawai et al. 2005; Ni-Ni-Win et al. 2011; Silberfeld et al. 2011; Tan et al. 2013). The current trend for floristic surveys, especially those involving taxa with simple or convergent morphologies, employed molecular techniques for more accurate identification (e.g., Cianciola et al. 2010; Kucera and Saunders 2012).

Our study aims to identify and document species of crustose brown algae in the Indo–Malay region by combining molecular data (using *rbc*L and partial *cox*1 sequences) and morphological observations, thus contributing to an improved understanding of the taxonomy, diversity and distribution of the tropical brown crusts from this region. Phylogenetic analyses of combined *rbc*L and *cox*1-5′ data were also conducted to infer the relationship among the identified crustose brown algal taxa.

Materials and methods

Collections of crustose brown algae were made from May 2009 to July 2012. Specimens from Peninsular Malaysia, Sabah (Borneo) and Lombok Island, Indonesia (Fig. 1) were collected in the field and air-dried prior to desiccation in silica gel. Voucher specimens were deposited in University of Malaya Seaweeds and Seagrasses Herbarium (KLU) while the culture strain of *Mesospora negrosensis* West et Calumpong was obtained from the Kobe University Macroalgal Culture Collection (KU-MACC) and used for DNA extraction. Eighty-seven crustose brown algal specimens were examined morphologically and sequenced using chloroplast *rbc*L and mitochondrial *cox*1-5′ molecular markers.

For anatomical studies, squash preparations of the brown crusts mounted on glass slides in corn syrup were observed under light microscope. Photomicrographs were taken using a DP72 digital camera attached to a BX51 microscope (Olympus, Japan).

Total DNA extractions were performed on ground tissue samples using the i-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology Inc., South Korea) following the manufacturer's instructions. Parameters for polymerase chain reaction (PCR) amplification and sequencing followed Poong et al. (2013). Primers used for *rbc*L amplification included: rbcFO, rbcF4 and rbcR2 (Kawai and Sasaki 2004); PRBF2, PRBF3, PRBR2, PRBR3 and RSPR (Kogame et al. 1999); NDrbcL2 and NDrbcL9 (Daugbjerg and Andersen 1997); RalR952 (Lim et al. 2007). RspBF2 (5′-TACGGTCGTGTTG TTTATGA-3′) and RspBR2 (5′-AGTCGCACCTGATTGAAT AC-3′) were newly designed for this study. Primers used for *cox*1-5′ amplification were 117F and 784R (Bittner et al. 2008); GazF2 and GazR2 (Lane et al. 2007) and L and H (Folmer et al. 1994). Amplification and sequencing of the *cox*1-5′ region was also conducted for some of the crustose brown algal taxa published in the study by Lim et al. (2007). PCR products were purified using LaboPass Gel & PCR purification kit (Cosmo Genetech, South Korea) while sequencing was undertaken by First Base Laboratories (Malaysia) with the same primers used for PCR amplification.

For molecular phylogenetic analyses, raw sequences were first assembled and edited via ChromasPro ver. 1.42 (Technelysium Pty. Ltd.), subsequently aligned using ClustalX v. 2.0.8 (Larkin et al. 2007) and then manually adjusted with Bioedit v. 7.0.9.0 (Hall 1999). Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were performed for each gene separately, and for the two genes combined. Combined analyses included 65 brown algal taxa plus three outgroup taxa (*Schizocladia ischiensis*, *Phaeothamnion confervicola* and *Tribonema aequale*) or only the 51 taxa (including *T. aequale* as outgroup) for which the sequences of both genes were determined to explore the influence of missing data. Crustose brown algal taxa used for molecular phylogenetic analyses of which sequences were newly generated for this study is listed in Table 1. Accession numbers of previously published taxa (both crustose brown and non-crustose brown) are given next to the species name in the combined ML phylogenetic tree (Fig. 2). Analyses of *rbc*L alignment alone included 68 taxa (using similar outgroups as the combined data set) and of *cox*1-5′ alignment alone, 50 taxa (including *Ishige okamurae* as outgroup). *I. okamurae* was used as outgroup in the *cox*1-5′ only analyses to improve resolution

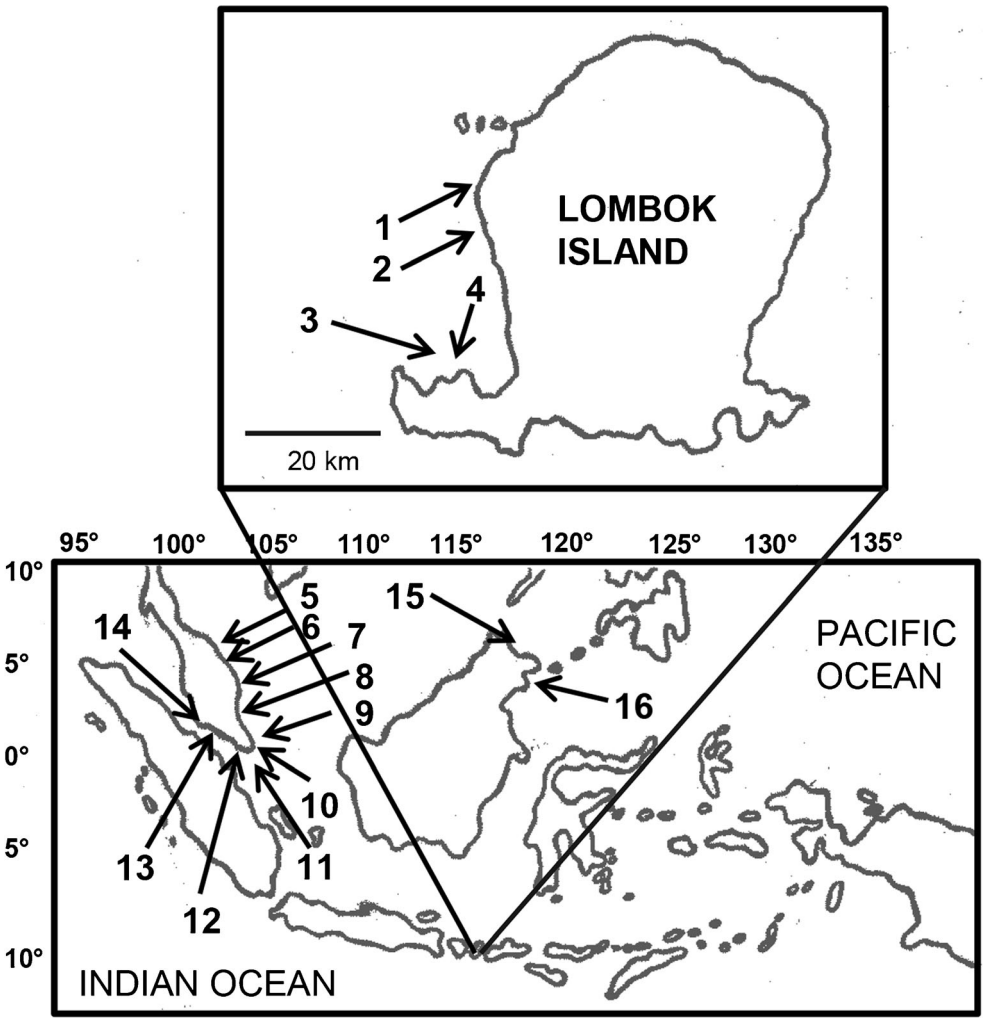

Fig. 1 Map indicating the collection sites of the specimens used in the present study (adapted from http://www.fao.org/docrep/field/009/ag160e/AG160E09.htm). *1* Nipah; *2* Batulayar; *3* Gili Genting; *4* Batukijok; *5* Pantai Chendering; *6* Pantai Kemasik; *7* Telok Kalong; *8* Teluk Sari; *9* Teluk Ramunia; *10* Pulau Che Kamat; *11* Pelabuhan Tanjung Langsat; *12* Pulau Merambong; *13* Pulau Besar; *14* Port Dickson; *15* Kampong Dandulit; *16* Semporna

within the brown algal taxa. The resulting phylogenies were screened for significant topological incongruency (conflicting relationships with supported nodes) to assess the feasibility of combining sequences from the two genes. The separate trees did not show any supported conflicting nodes, thus the focus was placed on the combined data set.

MP trees were constructed using PAUP 4.0b10 (Swofford 2002) under a heuristic search with 100 random sequence addition replicates and a tree bisection reconnection (TBR) branch-swapping algorithm with gaps treated as missing data. Bootstrap percentage (BP) was computed under a heuristic search method and TBR swapping with 1,000 replications and one random taxon additions to assess branch support.

Kakusan v.3 (Tanabe 2007) was used to determine the best-fit nucleotide substitution models for ML and BI analyses selected using the corrected Akaike information criterion (Akaike 1973) and the Bayesian information criterion (Schwarz 1978), respectively. ML trees were inferred using Treefinder v. October 2008 (Jobb et al. 2004) with BP generated from 1,000 resamplings to estimate robustness. BI analyses were conducted using MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003), and the program was set to run four chains of Markov chain Monte Carlo iterations for 2,000,000 generations, keeping one tree for every 100 generations. The first 2,000 trees sampled were discarded as "burn-in" to ensure stabilization, based on the stationarity of log likelihood values in the first 100,000 generations as assessed using Tracer v.1.5 (http://tree.bio.ed.ac.uk/software/tracer/). The remaining trees were used to compute a consensus topology and calculate the posterior probability (PP) values. For the purpose of comparison with bootstrapping, nodes with Bayesian PP >0.95 (the node appears in greater than 95% of sampled trees) are implied as being strongly supported, between 0.90 and 0.95 as moderately supported, and <0.90 as weakly supported. ML BP and MP BP are described as strong (≥85%), moderate (70–85%) and weak (<70%). To assess the levels of intra- and interspecific variations in the *rbc*L and *cox*1-5′ sequences, uncorrected (*p*)

Table 1 List of crustose brown algal specimens used in the molecular phylogenetic analyses of which sequences were newly generated for this study (accession numbers in bold)

Taxa	Collection site, date of collection, voucher number or reference of *rbc*L/*cox*1-5′ sequences	Genbank accession number *rbc*L/*cox*1-5′
Diplura simplex Tanaka et Chihara	Lim et al. 2007/this study	AB250084/**KC847385**
Diplura sp. B	Lim et al. 2007/this study	AB250086/**KC847386**
Diplura sp. F	Pantai Dickson, Malaysia; 16 Dec. 2009; PSM12208	**KC847395/KC847374**
Diplura sp. F	Gili Genting, Lombok Island, Indonesia; 10 June 2010; PSM12222	**KC847396/KC847375**
Diplura sp. F	Pantai Chendering, Terengganu, Malaysia; 16 Feb. 2012; PSM12325	**KC847397/KC847376**
Diplura sp. F	Semporna, Sabah, Malaysia; 5 July 2012; PSM12359	**KC847398/KC847377**
Diplura sp. G	Pulau Che Kamat, Johor, Malaysia; 29 May 2009; PSM12172	**KC847399/KC847378**
Diplura sp. G	Gili Genting, Lombok Island, Indonesia; 10 June 2010; PSM12224	**KC847400/KC847379**
Diplura sp. G	Batulayar, Lombok Island, Indonesia; 8 June 2010; PSM12215	**KC847401/KC847380**
Diplura sp. G	Pantai Dickson, Malaysia; 30 July 2012 ; PSM12371	**KC847402/KC847381**
Mesospora schmidtii Weber-van Bosse	Telok Kalong, Terengganu, Malaysia; 17 Feb. 2012; PSM12317	**KC847387/KC847366**
Mesospora schmidtii	Semporna, Sabah, Malaysia; 1 July 2012; PSM12353	**KC847388/KC847367**
Mesospora sp. C	Lim et al. 2007/This study	AB250065/**KC847382**
Mesospora negrosensis West et Calumpong	KU1065	**KC847389/KC847368**
Mesospora negrosensis	Pulau Merambong, Johor, Malaysia; 24 August 2009; PSM12183	**KC847390/KC847369**
Mesospora negrosensis	Pantai Chendering, Terengganu, Malaysia; 16 Feb. 2012; PSM12326	**KC847391/KC847370**
Neoralfsia expansa (J. Agardh) Lim et Kawai ex Cormaci et G. Furnari	Lim et al. 2007/This study	AB250077/**KC847383**
Neoralfsia expansa	Lim et al. 2007/This study	AB250078/**KC847384**
Neoralfsia expansa	Pulau Besar, Melaka, Malaysia; 11 April 2010; PSM12254	**KC847392/KC847371**
Neoralfsia expansa	Gili Genting, Lombok Island, Indonesia, 10 June 2010; PSM12230	**KC847393/KC847372**
Neoralfsia expansa	Pantai Kemasik, Terengganu, Malaysia; 17 Feb. 2012; PSM12322	**KC847394/KC847373**

PSM and KU indicate reference code of vouchers at the University of Malaya Seaweeds and Seagrasses Herbarium (KLU) and of culture obtained from Kobe University Macroalgal Culture Collection (KU-MACC), respectively

pairwise genetic distances were estimated using PAUP 4.0b10 (Swofford 2002).

Results

Molecular phylogenetic analyses

The length of the *rbc*L, *cox*1-5′ and combined alignments were 1,349, 665 and 2,014 nucleotides (nt), respectively. The *rbc*L gene was successfully sequenced for all 87 specimens (100% success).The *rbc*L intraspecific genetic variation ranged from 0–0.075% in *M. elongata* to 0–4.67% in *N. expansa*, while interspecific variation ranged from 2.99% between *D. simplex* and *Diplura* sp. C to 17.96–18.41% between *Diplura* sp. G and *Diplura* sp. B. Amplification of *cox*1-5′ gene was successful for all but four specimens (95% success). The *cox*1-5′ intraspecific divergences ranged from 0–0.149% in *M. elongata* to 0–15.35% in *M. schmidtii* while interspecific divergences ranged from 0.149% between *D. simplex* and *Diplura* sp. B to 18.48–22.45% between *M. negrosensis* and *M. schmidtii*. All three methods of phylogenetic inference (ML, MP, BI) resulted in near-identical trees for all well-supported nodes for the four data sets (genes combined or separate, and all taxa included or a subset). Nonetheless, the concatenated data gave better resolution and clade support than each individual gene. Phylogenetic signal was virtually congruent between the more variable *cox*1-5′ gene and the conserved *rbc*L gene and mainly carried by chloroplastic information. Results indicated that missing *cox*1-5′ sequences in the combined data set did not affect the overall phylogeny with variations only in the position of certain clades with low or no support; thus, the ML tree inferred from the combined data set with all taxa included is depicted with support (Fig. 2).

The combined data set included 2,014 characters, of which 1,201 (60%) were variable sites and 1,019 (51%) were parsimony-informative sites. MP analysis resulted in four equally most parsimonious trees, and tree length was 8,178, consistency index (CI) was 0.2539 and retention index (RI) was 0.5444. The ML and BI trees were constructed using a GTR + gamma and SYM + gamma models, respectively. The topologies obtained in all three analyses (ML, MP and BI) were reasonably congruent at the interordinal level, although there was little resolution of

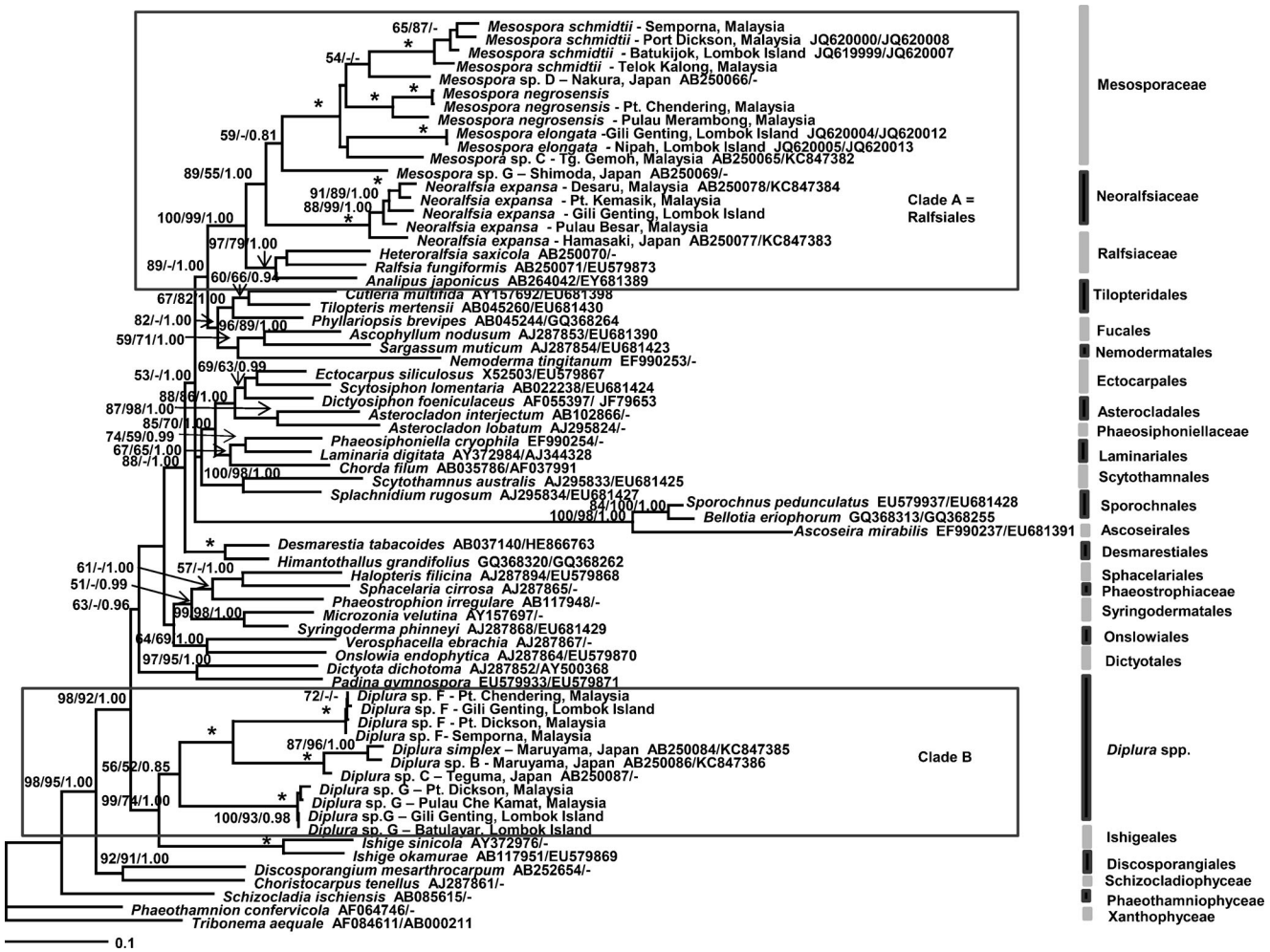

Fig. 2 ML phylogeny inferred based on the combined *rbc*L and partial *cox*1 data set. Numbers above each branch denote BP for ML (*left*), MP (*middle*) and PP for BI (*right*). *Asterisks* indicate 100% BP and 1.00 PP. *Dashes* indicate percentages of <50% (or that the node did not occur in the MP or BI tree). The –ln likelihood was 35,367.35; gamma distribution shape parameter (alpha)=0.3025804, nucleotide frequencies: *A*=0.25, *C*=0.25, *G*=0.25, *T*=0.25; and substitution model rate matrix: [TC=0.3666329, TA=0.2089154, TG=0.05988139, CA=0.0491223, CG=0.05342058, AG=0.2620275]. Scale bar=0.1 substitutions per site. Genbank accession numbers are given next to the species names for further information on the published taxa

relationships among the orders. The monophyly of all the brown algal orders was strongly to fully supported in all analyses (BP=98% [ML], 95% [MP]; PP =1.00).

For the *rbc*L only data set, 687 (51%) sites were variable and 546 (40%) sites were parsimony-informative out of the total 1,349 nucleotides. The number of parsimonious trees obtained was 28, the tree length was 4,605, and CI and RI indices were 0.2512 and 0.5695, respectively. As for the *cox*1-5′ data set, 509 (77%) nucleotides were variable and 469 (71%) nucleotides were parsimony-informative. Four most parsimonious trees were obtained, and tree length was 3,609, the CI index was 0.2502 and the RI index was 0.4849. Phylogenetic analyses using *rbc*L data gave a satisfactory resolution at the ordinal and familial level, whereas the use of *cox*1-5′ data alone was better suited for phylogeny inference at the species level. Intra- and interordinal relationships were poorly resolved in the *cox*1 trees which were within expectation as the mitochondrial-encoded gene was noted for its high evolutionary rate. This study also illustrated the feasibility of using *cox*1-5′ as a barcode marker for species of crustose brown algae.

Three species of *Mesospora* (*M. schmidtii*, *M. elongata* and *M. negrosensis*), two putative species of *Diplura* (*Diplura* sp. F and *Diplura* sp. G) and the monotypic genus, *N. expansa* were identified from the molecular analyses of the 87 specimens examined for this study (Table 2). *M. schmidtii* was the most common brown crusts found with 44 specimens, *M. negrosensis* (8), *M. elongata* (4), *N. expansa* (8), *Diplura* sp. F (14) and *Diplura* sp. G (9). The crustose brown algal taxa were not monophyletic but separated into two major clades (A and B). Clade A, which has strong to full support (BP=100% [ML], 99% [MP]; PP=1.00), corresponded to the order Ralfsiales and comprised of the families Mesosporaceae, Neoralfsiaceae and Ralfsiaceae. The family Mesosporaceae comprising *Mesospora*

Table 2 Floristic results of the six species identified in this study with information on the range of distribution and number of sequences

Species (number of specimens)	Range of collection (number of specimens per site)	Number of sequences	
		*rbc*L	*cox*1-5′
Mesospora schmidtii (*n*=44)	CHE (4), MER (10), POR (5), PTL(1), BAT (1), DAN (2), TKA (2), TKS (3), TKR (1), SEM (15)	44	44
Mesospora elongata (*n*=4)	NIP (1), GIL (2), BAT (1)	4	4
Mesospora negrosensis (*n*=8)	CHE(1), MER (2), CHD (3), SEM (2)	8	8
Neoralfsia expansa (*n*=8)	NIP (1), LAY(1), GIL(3), BES(1), KEM(1), CHE (1)	8	7
Diplura sp. F (*n*=14)	MER (1), POR (6), GIL (1), CHD (3), TKS (2), SEM (1)	14	11
Diplura sp. G (*n*=9)	CHE (1), MER (1), POR (3), LAY (2), GIL (1), SEM (1)	9	9

CHE Pulau Che Kamat, Johor, Malaysia; *MER* Pulau Merambong, Johor, Malaysia; *POR* Port Dickson, Malaysia; *PTL* Pelabuhan Tanjung Langsat, Johor, Malaysia, *SEM* Semporna, Sabah, Malaysia; *DAN* Kampong Dandulit, Sabah, Malaysia; *BES* Pulau Besar, Melaka, Malaysia; *CHD* Pantai Chendering, Terengganu, Malaysia; *KEM* Pantai Kemasik, Terengganu, Malaysia; *TKA* Telok Kalong, Terengganu, Malaysia; *TKS* Teluk Sari, Johor, Malaysia; *TKR* Teluk Ramunia, Johor, Malaysia; *NIP* Nipah, Lombok Island, Indonesia; *BAT* Batukijok, Lombok Island, Indonesia; *GIL* Gili Genting, Lombok Island, Indonesia; *LAY* Batulayar, Lombok Island, Indonesia

sp. C, *Mesospora* sp. D and the three species of *Mesospora* identified in the present study was resolved with maximum BP and PP when *Mesospora* sp. G, suspected to be a close relative of the genus, is omitted. Each of the *M. schmidtii*, *M. elongata* and *M. negrosensis* clades was resolved with full BP and PP support. The Mesosporaceae formed a sister relationship to the Neoralfsiaceae, and both were in turn resolved as sister to the Ralfsiaceae. Species of *Diplura* represented by Clade B, formed a sister relationship to Ishigeales with moderate to strong support (BP=99% [ML], 74% [MP]; PP=1.00), near the basal end of the phylogenetic tree. Surprisingly, *Diplura* sp. F is more closely related to *Diplura* spp. from Japan (BP=100% for both ML and MP; PP=1.00) than to *Diplura* sp. G in spite of their geographical distribution. Each of the *Diplura* sp. F and *Diplura* sp. G clades was resolved with full BP and PP support. However, the branch support for inclusion of all *Diplura* spp. was rather weak (BP=56% [ML], 52% [MP]; PP=0.85).

Morphological and anatomical observations

The diagnostic morpho-anatomical features of the crustose brown algae examined in the present study are summarised in Table 3. Species of *Mesospora* are generally characterized by their gelatinous thallus, loose organisation of vegetative filaments, single chloroplast in each cell, unilocular reproductive structures unaccompanied by paraphyses and plurilocular reproductive structures terminated by more than one sterile cell. At the species level, *Mesospora* spp. are distinguished based on the organisation of reproductive structures (Fig. 3a–f). The crusts of *N. expansa* are characteristically thicker than *Mesospora* and *Diplura* and a distinct delineation of the cortical and medullary layers are observed (Fig. 4a, b). Species of *Diplura* are recognised by their relatively thin thallus, multiple chloroplasts per cell and plurilocular structures terminated by a single sterile cell (Fig. 5a–c). The two putative species of *Diplura*, i.e., *Diplura* sp. F and *Diplura* sp. G, are barely distinguishable morphologically although unilocular reproductive structures were observed in *Diplura* sp. G (Fig. 5d) but not in *Diplura* sp. F. Morpho-anatomical characteristics of previously described *Diplura* spp. are included in Table 3 for comparison purpose.

Discussion

Our study indicates that the common crustose brown algae in the Indo–Malay region are species of *Mesospora*, *Diplura* and *Neoralfsia*. Despite global reports of crustose brown algae from the northern to southern hemisphere (e.g., Jaasund 1965; Fletcher 1987; Womersley 1987), the distribution of certain genera, or even species, are probably restricted to the colder ocean waters in the temperate, subpolar or subtropical regions. Members of the Ralfsiaceae (e.g., *Ralfsia fungiformis* (Gunnerus) Setchell et Gardner, *Analipus japonicus* (Harvey) Wynne and *Heteroralfsia saxicola* (Okamura et Yamada) Kawai; Fig. 2) which have been reported mostly from the temperate or colder water region (e.g., North America: Hollenberg 1969; Japan: Tanaka and Chihara 1980) were not encountered in our study. Species of *Mesospora* are the more common brown crusts found in this region surrounded by the warm waters of the eastern Indian Ocean and the South China Sea (e.g., West and Calumpong 1996; Krishnamurthy and Baluswami 1986). In contrast, Japan and Hong Kong recorded a high diversity of crustose brown algal taxa (e.g., Kaehler 1998; Tanaka and Chihara 1980) presumably due to the influence of the Pacific Ocean and the outcome of a dedicated study to this group of algae. *N. expansa* (previously known as *Ralfsia expansa*) have a cosmopolitan distribution and was reported in almost all continents (e.g., León-Alvarez and González-González 2003; Ribera et al. 1992; Rull Lluch 2002).

Table 3 Comparison of morphological characters among (1) genera and (2) species of crustose brown algae in the present study

Taxa	Relative comparison of thalli thickness and gelatinous feature (especially in fertile thallus)	Organisation of vegetative filaments	Distinct delineation of cortical and medullary layer	Plurilocular reproductive structures	Unilocular reproductive structures	Number of chloroplasts per cell
Mesospora	Thicker than *Diplura* but thinner than *Neoralfsia*. Gelatinous upon contact with water	Loosely adherent, with exception in certain species. Cell numbers of the vegetative filaments can be used for species level identification	No	Presence or absence is species dependent. Generally more common than unilocular reproductive structures	Presence or absence is species dependent. Position of the structure on the erect filament and number of stalk cells is useful for species level identification.	Single
M. schmidtii	Thicker than *Diplura* but thinner than *Neoralfsia*. Gelatinous upon contact with water	Loosely adherent, held together by the gelatinous matrix and joined at the basal portion	No	Initially uniseriate later biseriate, and terminated by 2–3 terminal cells (Fig. 3a)	Terminally inserted on up to 4 stalk cells, lateral and basal to the surrounding filaments. Paraphyses lacking (Fig. 3d)	Single
M. negrosensis	Thicker than *Diplura* but thinner than *Neoralfsia*. Gelatinous upon contact with water	Rather closely adherent especially the lower half portion of the vegetative filaments	No	Generally uniseriate, sometimes biseriate; terminated by 1–3 (usually 2) enlarged sterile terminal cells (Fig. 3b)	Terminally inserted on 1–2 stalk cells, lateral and basal to the surrounding filaments. Paraphyses lacking (Fig. 3e)[a]	Single
M. elongata	Thicker than *Diplura* but thinner than *Neoralfsia*. Gelatinous upon contact with water	Loosely adherent, held together by the gelatinous matrix and joined at the basal portion	No	Initially uniseriate later biseriate, and terminated by 2–4 terminal cells (Fig. 3c)	Terminally inserted on up to 8 stalk cells, lateral and basal to the surrounding filaments. Paraphyses lacking (Fig. 3f)	Single
Neoralfsia expansa (monotypic genus)	Thick crusts. Gelatinous upon contact with water (Fig. 4a)	Filaments are tightly adherent	Yes	Biseriate, terminated by a sterile terminal cell. Less common than unilocular structures	Terminally inserted on 3–6 stalk cells at the terminal end of erect filaments. Paraphyses are present (Fig. 4b)	Single
Diplura	Very thin. Slightly gelatinous upon contact with water (Fig. 5a)	Filaments are somewhat tightly adherent	No	Present in all species reported with varying structures among species	Species dependent. Not observed in the type species	Multiple
Diplura sp. F	Smooth and thin. Slightly gelatinous upon contact with water	Filaments are somewhat tightly adherent	No	Initially uniseriate later biseriate, and both filaments shared a sterile terminal cell (Fig. 5b)	Not observed	Multiple
Diplura sp. G	Smooth and thin. Slightly gelatinous upon contact with water	Filaments are somewhat tightly adherent	No	Initially uniseriate later biseriate, and both filaments shared a sterile terminal cell (Fig. 5c)	Terminally inserted on two stalk cells, lateral to the surrounding filaments. Paraphyses are present (Fig. 5d)	Multiple
D. simulans[b]	Gelatinous	Loosely held together by gelatinous matrix and readily separating under pressure	No	Uniseriate, single or mostly in pairs with a sterile terminal cell at the apex	Unknown and probably lacking	Several to many
D. simplex[c,d]	Smooth and thin, somewhat gelatinous	Tightly adherent, not so readily separated	No	Standing in two rows on each erect filament, reproductive filament biseriate bearing one sterile terminal cell	Terminally inserted on one to two stalk cells. Paraphyses are present	Several
Diplura sp. B[d]	Somewhat thin	Tightly adherent	No	Mostly uniseriate, single sterile terminal cell	Unknown	Several
Diplura sp. C[d]	Somewhat thin	Tightly adherent	No	Mostly uniseriate, single sterile terminal cell	Unknown	Several

Table 3 (continued)

Taxa	Relative comparison of thalli thickness and gelatinous feature (especially in fertile thallus)	Organisation of vegetative filaments	Distinct delineation of cortical and medullary layer	Plurilocular reproductive structures	Unilocular reproductive structures	Number of chloroplasts per cell
Diplura sp. "*australis*" [e]	Less gelatinous	Laterally coherent, separate only with considerable pressure	No	Usually uniseriate and in pairs, each reproductive filament has a single pale coloured sterile terminal cell	Absent	Several

[a] First report of unilocular reproductive structures in *Mesospora negrosensis*
[b] Data from Hollenberg (1969) and Abbott and Hollenberg (1976)
[c] Data from Tanaka and Chihara (1981)
[d] Data from Lim et al. (2007)
[e] Data from Buchanan (2005)

The crustose brown algae found in Malaysia and Lombok Island included *M. schmidtii*, *N. expansa*, *Diplura* sp. F and *Diplura* sp. G. Conversely, *M. negrosensis* was found in Malaysia but not on Lombok Island, and it was vice versa for *M. elongata*. A relatively higher diversity of crustose brown algae was recorded at the south-western and north-eastern parts of Peninsular Malaysia and the western part of Lombok Island. The specimens were predominantly epilithic in the intertidal zone, although some of the *N. expansa* crusts were epizoic and were found in the subtidal zone. This is the first report of *M. negrosensis* from Malaysia. This species has thus far been reported only from the Philippines (West and Calumpong 1996). DNA sequencing of *rbc*L and partial *cox*1 genes from our specimens matched those of the culture strain of *M. negrosensis* deposited in KU-MACC. Apart from that, the presence of *Diplura* spp. in Malaysia and Indonesia is also documented for the first time here, expanding the known range for this genus. Other members of this genus, i.e., *D. simulans* Hollenberg and *D. simplex* Tanaka and Chihara, were previously reported in North America (Hollenberg 1969), Mexico (Pedroche et al. 2008), Japan (Tanaka and Chihara 1981; Lim et al. 2007) and Hong Kong (Kaehler 1994), while an unidentified species, *Diplura* sp. "australis" has been documented in New Zealand (Buchanan 2005).

The two species of *Stragularia* reported by Weber-van Bosse (1913) from Indonesia are potentially species of a different genus because members of the family Scytosiphonaceae are rarely, if ever, reported from warmer water regions. The description (Weber-van Bosse 1913) given for *S. clavata* is too brief and incomplete for definite identification, whereas for *S. polycarpa*, the thallus construction and unilocular reproductive structures described and illustrated bear a slight resemblance to those of *Diplura* sp. G. However, four to five sporangia inserted on one or two stalk cells were described for *S. polycarpa* as opposed to one to two sporangia on a single stalk cell in *Diplura* sp. G. Furthermore, plurilocular reproductive structures, which are common in *Diplura* spp., were not observed in *S. polycarpa*. To our knowledge, there is no further mention of *S. polycarpa* in the literature since the first report by Weber-van Bosse (1913). Therefore, we refrain from making any conclusions on the taxonomic status of these two taxa, especially considering that they were initially reported from other locations in Indonesia which do not include Lombok Island.

Mesospora is regarded as a synonym of *Hapalospongidion* (Womersley 1987), but Poong et al. (2013) retained them as distinct genera pending molecular data from the generitype. The three genera identified in this study are distinguished based on thickness of thallus/crust, organization of vegetative filaments, reproductive structures and number of chloroplasts (Table 3). The thickness of crust decreased in the order of *Neoralfsia* > *Mesospora* > *Diplura*. Vegetative filaments in *Mesospora* spp. are generally loosely adhered to each other and are readily separated by slight pressure. Erect filaments in *Diplura* spp. are

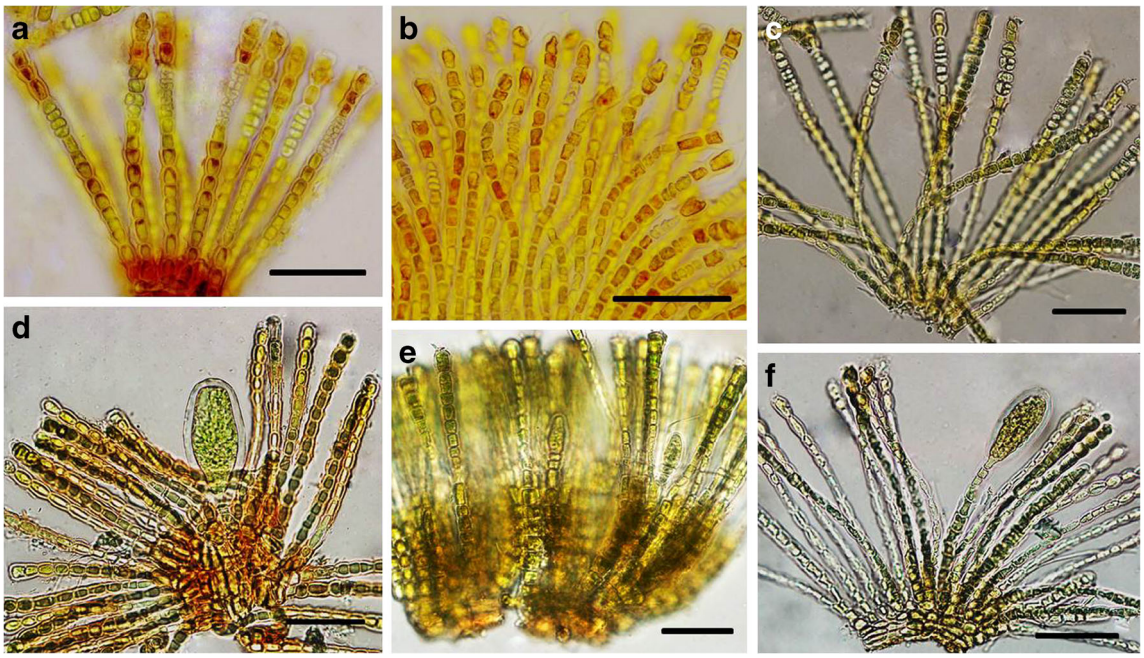

Fig. 3 *Mesospora* spp. **a–c** Plurilocular reproductive structures of *M. schmidtii* (voucher number: PSM 12203), *M. negrosensis* (voucher number: PSM 12183) and *M. elongata* (voucher number: PSM 12214) borne near the apex of erect filaments with sterile terminal cells, respectively. Scale bars=50 μm. **d–f** Unilocular reproductive structures of *M. schmidtii* (voucher number: PSM 12353), *M. negrosensis* (voucher number: PSM 12324) and *M. elongata* (voucher number: PSM12221) inserted lateral to the surrounding erect filaments, respectively. Scale bars=50 μm

more tightly adhered and are only partially separated by pressure whereas in *N. expansa*, erect filaments are tightly adhered to each other and are difficult to be separated even by applying pressure. *Mesospora* and *N. expansa* are reported to have a single chloroplast per cell while *Diplura* is known for its multiple chloroplasts per cell. Plurilocular reproductive structures are more commonly observed compared to unilocular reproductive structures for both *Mesospora* and *Diplura* but not for *N. expansa*.

Three species of *Mesospora* were collected in this study, and they can be distinguished based on several features. *M. schmidtii*, which is the type, differed from *M. elongata* in their number of cells and the number of stalk cells associated with the unilocular reproductive structures. The plurilocular reproductive structures of *M. negrosensis* are generally uniseriate, and its sterile terminal cells are characteristically enlarged. Unilocular reproductive structures for *M. negrosensis* were observed for the first time in this study although they were not completely matured. A recent study by Poong et al. (2013) compiled a detailed comparison of morpho-anatomical features among species of *Mesospora*.

Two species of *Diplura*, tentatively designated as *Diplura* sp. F and *Diplura* sp. G, were identified in our study, and unilocular reproductive structures were observed for *Diplura* sp. G. Hollenberg (1969) and Tanaka and Chihara (1981) did not observe unilocular reproductive structures for *D. simulans* and *D. simplex*, and these structures were first mentioned in *D. simplex* by Lim et al. (2007). Tanaka and Chihara (1981) proposed and distinguished *D. simplex* from *D. simulans* on the basis of the size, thickness and construction of the crusts. Although it is uncertain whether Tanaka and Chihara examined the authentic specimen of *D. simulans*, these features seemed

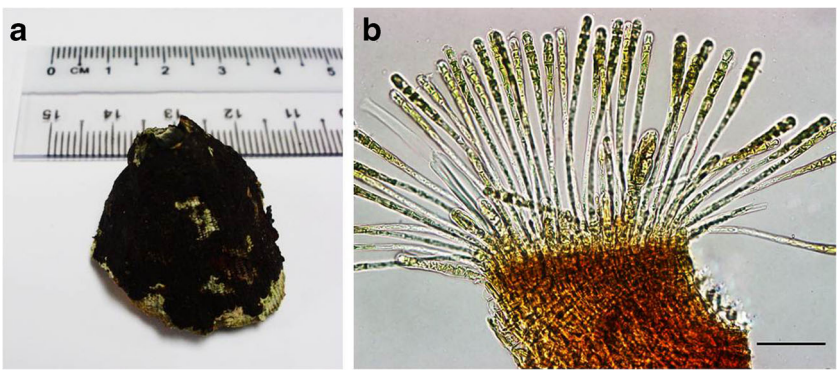

Fig. 4 *Neoralfsia expansa*. **a** Thallus in the form of thick crusts loosely adherent on rocks (voucher number: PSM 12322). **b** Unilocular reproductive structures with stalk cells among surrounding erect filaments (voucher number: PSM 12223). Scale bar=50 μm

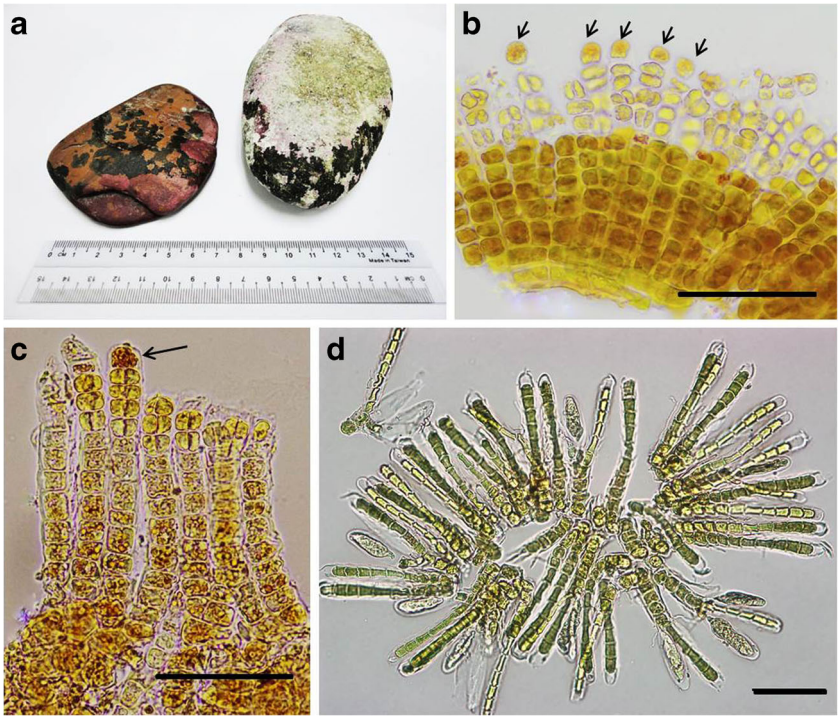

Fig. 5 *Diplura* spp. **a** Thin crusts growing on rocks (*left*: *Diplura* sp. G, voucher number: PSM 12193; *right*: *Diplura* sp. F, voucher number: PSM 12359). **b** Plurilocular reproductive structures of *Diplura* sp. F (voucher number: PSM 12208) terminated by a sterile terminal cell (marked with an arrow). Scale bar=50 μm. **c** Plurilocular reproductive structures of *Diplura* sp. G (voucher number: PSM 12224) terminated by a sterile terminal cell (marked with an arrow). Scale bar=50 μm. **d** Unilocular reproductive structures of *Diplura* sp. G (voucher number: PSM 12172) on stalk cells growing lateral to surrounding erect filaments. Scale bar=50 μm

insufficient for species delineation, and sequence data of *D. simulans* from the type locality is necessary for confirmation. Molecular sequencing allowed the separation of *Diplura* sp. F and *Diplura* sp. G when no single outstanding morphological feature is available to distinguish them despite the wide genetic differences (*rbc*L interspecific distance=12.92–15.66%; *cox*1 interspecific distance=19.37–20.42%). The low interspecific variation between *D. simplex* and the two undescribed species, *Diplura* sp. B and *Diplura* sp. C [*p* distance=3.36–5.27% (*rbc*L); 0.15% (*cox*1)] leads us to speculate that the three are probably conspecific.

Molecular data is essential in the notoriously challenging identification of crustose brown algae. Some species are stages of other taxa with heteromorphic life histories, particularly in the Scytosiphonaceae (Kain et al. 2010). The conventional method of identification up to genus and species level is based on the construction of thalli, life history patterns, number of chloroplasts, occurrence of sessile or stalked unilocular reproductive structures associated with multicellular paraphyses and plurilocular reproductive structures with sterile terminal cells. Although the position and organization of the reproductive structures are crucial for positive identification, collection of fertile specimens is often by chance, especially in the tropics where seasonality is not observed. Variation of terminology used by authors in describing the position of reproductive structures further complicates the identification process (León-Alvarez and Norris 2005). Additionally, there is a risk of misidentification due to confluence of thalli from two or more different species.

Our molecular analyses involve a larger taxon sampling in which more brown algal orders were included compared to the study by Lim et al. (2007). Although a number of taxa are missing in *cox*1 data, we decided to include them in the combined analyses since a study by Wiens (2009) demonstrated that the addition of missing taxa to a data set can be highly beneficial and improve phylogenetic accuracy and cases of decreased accuracy are limited. Although the *cox*1 marker was ineffective at resolving interfamilial and interordinal relationships, it was capable of assigning samples to genetic species. Combination of *rbc*L and *cox*1-5′ data is advocated for use in species identification and phylogenetic reconstruction of this group of algae.

Lim et al. (2007) were the first to dedicate a molecular study to the Ralfsiales as a whole to test their traditional classification. Most specimens originated from Japan, with only two taxa from Malaysia. Their circumscription of Ralfsiales excluded the families Neoralfsiaceae and Lithodermataceae which, along with Ralfsiaceae, were initially included in the order (Nakamura 1972). Molecular evidence by Reviers et al. (2007; Fig. 14.5, p. 278) indicated that Ralfsiaceae (Ralfsiales), Nemodermataceae (Nemodermatales) and Lithodermataceae are not monophyletic. Our findings concur with earlier results (Lim et al. 2007; Reviers et al. 2007) which showed that the brown crusts are not monophyletic. Specimens used in the present study were resolved in two major clades, clade A corresponding to the Ralfsiales and clade B which encompassed the *Diplura* spp. and which diverged much earlier and form a sister clade to the Ishigeales. Our circumscription of the Ralfsiales followed the approach used

by Lim et al. (2007) which included only the Ralfsiaceae, Mesosporaceae and Neoralfsiaceae.

Although the establishment of a new family as suggested by Lim et al. (2007) is necessary to accommodate species of *Diplura*, it is premature to do so at this stage as we await the publication of the gene sequences of the generitype, *D. simulans*. Further investigation of morpho-anatomical characters (and life history studies, if necessary) will help in understanding the evolutionary history of this early diverging group. As of now, the placement of this genus among the early lineages of brown algae is supported by the presence of several chloroplasts per cell. Putative *Diplura* spp. from Malaysia and Lombok Island displayed sister relationship with *D. simplex* and two other *Diplura* spp. from Japan. The phylogenetic relationship inferred for species of *Diplura* examined in this study mirrored the geographic location where these specimens were collected, i.e., samples from Japan, collectively formed a sister clade to Indo-Malaysian samples. *Diplura* sp. G, which was resolved as a sister to *Diplura* sp. F and Japanese *Diplura* specimens, may represent a separate but closely related genus; nonetheless, current data are insufficient to support this hypothesis.

More work is necessary on crustose brown algae, in particular sampling of genera that were previously assigned to the Ralfsiales such as *Jonssonia* Lund, *Acrospongium* Schiffner, *Symphyocarpus* Rosenvinge, *Sorapion* Kuckuck, *Zeacarpa* Anderson, Simons and Bolton and *Basispora* John and Lawson, for molecular studies in order to clarify their ambiguous taxonomic position. We anticipate the discovery of more crustose brown algal species from this region following the exposure to DNA sequencing, subsequently altering the makeup of the diversity of this under-represented group of brown algae as it was previously known from morphological descriptions.

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